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********** Welcome to STN International **********
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FILE 'MEDLINE' ENTERED AT 15:18:50 ON 23 DEC 1998
E TELOMERASE/CT
E E3+ALL
L1 706 S E24
L2 85160 S CLONING, MOLECULAR/CT
L3 19 S L1 AND L2
E RECOMBINANT PROTEIN/CT
L4 61110 S E4
L5 8 S L1 AND L4 NOT L3
L6 9 S HTERT
L7 5583 S TERT
L8 19 S L1 AND L2
L9 0 S L8 NOT (L3 OR L6)

E# FREQUENCY AT TERM
-- --------- -- ----
E1 6 TELLURIUM: UR, URINE/CT
E2 0 2 TELMIN/CT
E3 706 25 --> TELOMERASE/CT
E4 77 TELOMERASE: AI, ANTAGONISTS & INHIBITORS/CT
E5 95 TELOMERASE: AN, ANALYSIS/CT
E6 39 TELOMERASE: BI, BIOSYNTHESIS/CT
E7 7 TELOMERASE: BL, BLOOD/CT
E8 26 TELOMERASE: CH, CHEMISTRY/CT
E9 10 TELOMERASE: DE, DRUG EFFECTS/CT
E10 8 TELOMERASE: DF, DEFICIENCY/CT
E11 1 TELOMERASE: DU, DIAGNOSTIC USE/CT
E12 165 TELOMERASE: GE, GENETICS/CT

E1 0 BT9 D Chemicals and Drugs/CT
E2 0 BT8 Enzymes, Coenzymes, and Enzyme Inhibitors/CT
E3 14893 BT7 Enzymes/CT
E4 5951 BT6 Transferases/CT
E5 10614 BT5 Phosphotransferases/CT
E6 4160 BT4 Nucleotidyltransferases/CT
E7 4124 BT3 DNA Nucleotidyltransferases/CT
E8 5928 BT2 DNA-Directed DNA Polymerase/CT
E9 0 BT7 D Chemicals and Drugs/CT
E10 0 BT6 Amino Acids, Peptides, and Proteins/CT
E11 91531 BT5 Proteins/CT
E12 27400 BT4 Viral Proteins/CT
E13 1674 BT3 Retroviridae Proteins/CT
E14 0 BT9 D Chemicals and Drugs/CT
E15 0 BT8 Amino Acids, Peptides, and Proteins/CT
E16 91531 BT7 Proteins/CT
E17 27400 BT6 Viral Proteins/CT
E18 753 BT5 Viral Structural Proteins/CT
E19 29 BT4 Nucleocapsid Proteins/CT
E20 2481 BT3 Viral Core Proteins/CT
E21 261 BT2 Gene Products, pol/CT
E22 6655 BT1 RNA-Directed DNA Polymerase/CT
E23 706 --> Telomerase/CT
E24 706 MN D8.586.913.696.445.308.300.750.750./CT
EC EC 2.7.7.-
DC an INDEX MEDICUS major descriptor
NOTE Essential ribonucleoprotein reverse transcriptase that adds telomeric
DNA to the ends of eukaryotic chromosomes. Telomerase appears to be
repressed in normal human somatic tissues but reactivated in cancer, and
thus may be necessary for malignant transformation.
EC 2.7.7.-.
AQ AD AE AI AN BI BL CF CH CL CS CT DE DF DU EC GE HI IM IP ME PD
PH PK PO RESD SE ST TO TU UL UR
PNTE DNA Nucleotidylexotransferase (89-95)

HNTE 96
MHTH NLM 1996
********* END *********

L3 ANSWER 1 OF 19 MEDLINE
TI The reverse transcriptase component of the Tetrahymena telomerase ribonucleoprotein complex.

L3 ANSWER 2 OF 19 MEDLINE
TI Telomerase reverse transcriptase genes identified in Tetrahymena thermophila and Oxytricha trifallax.

L3 ANSWER 3 OF 19 MEDLINE
TI Extra-chromosomal telomere repeat DNA in telomerase-negative immortalized cell lines.

L3 ANSWER 4 OF 19 MEDLINE
TI Cloning and characterization of human and mouse telomerase RNA gene promoter sequences.

L3 ANSWER 5 OF 19 MEDLINE
TI Association of nucleoside diphosphate kinase nm23-H2 with human telomeres.

L3 ANSWER 6 OF 19 MEDLINE
TI Extension of life-span by introduction of telomerase into normal human cells [see comments].

L3 ANSWER 7 OF 19 MEDLINE
TI Telomerase gene identified [news].

L3 ANSWER 8 OF 19 MEDLINE
TI Programmed translational frameshifting in a gene required for yeast telomere replication.

L3 ANSWER 9 OF 19 MEDLINE
TI Coordinate regulation of G- and C strand length during new telomere synthesis.

L3 ANSWER 10 OF 19 MEDLINE
TI hEST2, the putative human telomerase catalytic subunit gene, is up-regulated in tumor cells and during immortalization.

L3 ANSWER 11 OF 19 MEDLINE
TI A mammalian telomerase component gene TLP1.

L3 ANSWER 12 OF 19 MEDLINE
TI Variable telomeric repeat synthesis in Paramecium tetraurelia is consistent with misincorporation by telomerase.

L3 ANSWER 13 OF 19 MEDLINE
TI A functional telomerase RNA swap in vivo reveals the importance of nontemplate RNA domains.

L3 ANSWER 14 OF 19 MEDLINE
TI TLP1: a gene encoding a protein component of mammalian telomerase is a novel member of WD repeats family.

L3 ANSWER 15 OF 19 MEDLINE
TI A mammalian telomerase-associated protein [see comments].

L3 ANSWER 16 OF 19 MEDLINE
TI Senescence mutants of Saccharomyces cerevisiae with a defect in telomere replication identify three additional EST genes.

L3 ANSWER 17 OF 19 MEDLINE
TI Cdc13p: a single-strand telomeric DNA-binding protein with a dual role in yeast telomere maintenance.

L3 ANSWER 18 OF 19 MEDLINE
TI Control of telomere growth by interactions of RAP1 with the most distal telomeric repeats.

L3 ANSWER 19 OF 19 MEDLINE
TI The fidelity of human telomerase.

L3 ANSWER 6 OF 19 MEDLINE
AN 1998113956 MEDLINE DN 98113956
TI Extension of life-span by introduction of telomerase into normal human cells [see comments].
CM Comment in: Science 1998 Jan 16;279(5349):334-5
AU Bodnar A G; Ouellette M; Frolkis M; Holt S E; Chiu C P; Morin G B; Harley C B; Shay J W; Lichtsteiner S; Wright W E
CS Geron Corporation, Menlo Park, CA 94025, USA.
NC AG07992 (NIA) AG05747 (NIA)
SO SCIENCE, (1998 Jan 16) 279 (5349) 349-52. Journal code: UJ7. ISSN: 0036-8075.
CY United States DT Journal; Article; (JOURNAL ARTICLE) LA English
FS Priority Journals; Cancer Journals EM 199804 EW 19980401
AB Normal human cells undergo a finite number of cell divisions and ultimately enter a nondividing state called replicative senescence. It has been proposed that telomere shortening is the molecular clock that triggers senescence. To test this hypothesis, two telomerase-negative normal human cell types, retinal pigment epithelial cells and foreskin fibroblasts, were transfected with vectors encoding the human telomerase catalytic subunit. In contrast to telomerase-negative control clones, which exhibited telomere shortening and senescence, telomerase-expressing clones had elongated telomeres, divided vigorously, and showed reduced
straining for beta-galactosidase, a biomarker for senescence. Notably, the telomerase-expressing clones have a normal karyotype and have already exceeded their normal life-span by at least 20 doublings, thus establishing a causal relationship between telomere shortening and in vitro cellular senescence. The ability to maintain normal human cells in a phenotypically youthful state could have important applications in research and medicine. CT Check Tags: Human; Support, U.S. Gov't, P.H.S. beta-Galactosidase: ME, metabolism
Biological Markers
Catalysis
*Cell Aging
*Cell Division
Cell Line
Cell Transformation, Neoplastic
* Cloning, Molecular*
Fibroblasts: CY, cytology
Homeostasis
Karyotyping
Phenotype
Pigment Epithelium of Eye: CY, cytology
Proteins: GE, genetics
*Proteins: ME, metabolism
RNA-Directed DNA Polymerase: GE, genetics
RNA-Directed DNA Polymerase: ME, metabolism
Stem Cells: CY, cytology
Stem Cells: EN, enzymology
* Telomerase: GE, genetics*
Telomerase: ME, metabolism
Telomere: ME, metabolism
*Telomere: PH, physiology
Telomere: UL, ultrastructure
Transfection
Tumor Cells, Cultured
CN EC 2.7.7.- (Telomerase); EC 2.7.7.49 (RNA-Directed DNA Polymerase); EC 3.2.1.23 (beta-Galactosidase); 0 (Biological Markers); 0 (Est2 protein); 0 (Proteins)

L3 ANSWER 7 OF 19 MEDLINE
AN 1998110868 MEDLINE DN 98110868
TI Telomerase gene identified [news].
AU Anonymous
SO ENVIRONMENTAL HEALTH PERSPECTIVES, (1997 Oct) 105 (10) 1043-4.
Journal code: EI0. ISSN: 0091-6765.
CY United States DT News Announcement LA English FS Priority Journals
EM 199803 EW 19980305
CT Check Tags: Human
* Cloning, Molecular*
*Neoplasms: GE, genetics
Telomerase: GE, genetics
CN EC 2.7.7.- (Telomerase)

L3 ANSWER 10 OF 19 MEDLINE
AN 97433088 MEDLINE DN 97433088
TI hEST2, the putative human telomerase catalytic subunit gene, is up-regulated in tumor cells and during immortalization.

AU Meyerson M; Counter C M; Eaton E N; Ellisen L W; Steiner P; Caddle S D; Ziaugra L; Beijersbergen R L; Davidoff M J; Liu Q; Bacchetti S; Haber D A; Weinberg R A
CS Whitehead Institute for Biomedical Research, Department of Biology, Massachusetts Institute of Technology, Cambridge 02142, USA.
NC CA 58596 (NCI) CA 39826 (NCI)
SO CELL, (1997 Aug 22) 90 (4) 785-95. Journal code: CQ4. ISSN: 0092-8674.
CY United States DT Journal; Article; (JOURNAL ARTICLE) LA English
FS Priority Journals; Cancer Journals OS GENBANK-AA281296; GENBANK-AF018167
EM 199712 EW 19971203
AB Telomerase, the ribonucleoprotein enzyme that elongates telomeres, is repressed in normal human somatic cells but is reactivated during tumor progression. We report the cloning of a human gene, hEST2, that shares significant sequence similarity with the telomerase catalytic subunit genes of lower eukaryotes. hEST2 is expressed at high levels in primary tumors, cancer cell lines, and telomerase-positive tissues but is undetectable in telomerase-negative cell lines and differentiated telomerase-negative tissues. Moreover, the message is up-regulated concomitant with the activation of telomerase during the immortalization of cultured cells and down-regulated during in vitro cellular differentiation. Taken together, these observations suggest that the induction of hEST2 mRNA expression is required for the telomerase activation that occurs during cellular immortalization and tumor progression.
CT Check Tags: Human; Male; Support, Non-U.S. Gov't; Support, U.S. [redacted]P.H.S.
[redacted] Acid Sequence
Catalysis
Cell Differentiation
*Cell Transformation, Neoplastic
Chromosome Mapping
* Cloning, Molecular*
Molecular Sequence Data
Protein Conformation
Proteins: CH, chemistry
*Proteins: GE, genetics
Sequence Alignment
* Telomerase: CH, chemistry*
Telomerase: GE, genetics
* Telomerase: ME, metabolism*
Testis: CH, chemistry
Transcription, Genetic
Tumor Cells, Cultured
*Up-Regulation (Physiology)
CN EC 2.7.7.- (Telomerase); 0 (Est2 protein); 0 (Proteins)

L3 ANSWER 11 OF 19 MEDLINE
AN 97359300 MEDLINE DN 97359300
TI A mammalian telomerase component gene TLP1.
AU [redacted]shikawa F; Nakayama J
[redacted]partment of Life Science, Tokyo Institute of Technology, Yokohama, Japan.
[redacted]ANPAKUSHITSU KAKUSAN KOSO. PROTEIN, NUCLEIC ACID, ENZYME, (1997 Jul) 42 (9) 1407-19. Ref: 21 Journal code: Q7D. ISSN: 0039-9450. CY Japan DT Journal; Article; (JOURNAL ARTICLE) General Review; (REVIEW) (REVIEW, TUTORIAL) LA Japanese EM 199712 EW 19971201
CT Check Tags: Animal
Base Sequence
* Cloning, Molecular*
*Lipoproteins: GE, genetics
*Membrane Proteins: GE, genetics
Molecular Sequence Data
Polymerase Chain Reaction: MT, methods
* Telomerase: CH, chemistry*
Telomerase: GE, genetics
* Telomerase: IP, isolation & purification*
*Tetrahymena: EN, enzymology
CN EC 2.7.7.- (Telomerase); 0 (Lipoproteins); 0 (Membrane Proteins); 0 (Plp1 protein)

L3 ANSWER 14 OF 19 MEDLINE
AN 97236507 MEDLINE DN 97236507
TI TLP1: a gene encoding a protein component of mammalian telomerase is a novel member of WD repeats family.
AU Nakayama J; Saito M; Nakamura H; Matsuura A; Ishikawa F
CS Department of Life Science, Tokyo Institute of Technology, Yokohama, Japan.
SO CELL, (1997 Mar 21) 88 (6) 875-84. Journal code: CQ4. ISSN: 0092-8674.
CY United States DT Journal; Article; (JOURNAL ARTICLE) LA English
FS Priority Journals; Cancer Journals EM 199706 EW 19970604
AB We have cloned and characterized the rat telomerase protein component 1 gene (TLP1), which is related to the gene for Tetrahymena p80. The cDNA encodes a 2629 amino acid sequence and produces the TLP1 proteins p240 and p230. The anti-TLP1 antibody specifically immunoprecipitated the telomerase activity. Moreover, p240 and p230 were copurified with telomerase activity in a series of extensive purification experiments. These results strongly suggest that the TLP1 proteins are components of, or are closely associated with, the rat telomerase. A pulse-chase experiment showed that p240 is modified to p230 in vivo. p230 was the dominant form in telomerase-positive cells, suggesting that modification of the TLP1 protein may regulate telomerase activity in vivo.
CT Check Tags: Animal; Human; Support, Non-U.S. Gov't
Blotting, Northern
Blotting, Western
* Cloning, Molecular*
COS Cells: PH, physiology
DNA, Complementary: AN, analysis
Gene Expression Regulation, Enzymologic: PH, physiology
Mammals
Mice
Molecular Sequence Data
Precipitin Tests
Rats
*Repetitive Sequences, Nucleic Acid
Sequence Homology, Amino Acid
Telomerase: GE, genetics
CN EC 2.7.7.- (Telomerase); 0 (DNA, Complementary)

E# FREQUENCY AT TERM
-- --------- -- ----
E1 0 2 RECOMBINANT MITOGEN-ACTIVATED PROTEIN KINASE P42 (MAPK)/CT
E2 0 2 RECOMBINANT PITUITARY GROWTH HORMONES/CT
E3 0 --> RECOMBINANT PROTEIN/CT
E4 61110 39 RECOMBINANT PROTEINS/CT
E5 0 2 RECOMBINANT PROTEINS, DNA/CT
E6 2 RECOMBINANT PROTEINS: AA, ANALOGS & DERIVATIVES/ CT
E7 2861 RECOMBINANT PROTEINS: AD, ADMINISTRATION & DOSAGE/CT
E8 1310 RECOMBINANT PROTEINS: AE, ADVERSE EFFECTS/CT
E9 60 RECOMBINANT PROTEINS: AG, AGONISTS/CT
E10 776 RECOMBINANT PROTEINS: AI, ANTAGONISTS & INHIBITORS/CT
E11 1393 RECOMBINANT PROTEINS: AN, ANALYSIS/CT
E12 11001 RECOMBINANT PROTEINS: BI, BIOSYNTHESIS/CT

L5 ANSWER 1 OF 8 MEDLINE
TI Myc activates telomerase.

L5 ANSWER 2 OF 8 MEDLINE
TI Telomerase activity is restored in human cells by ectopic expression of hTERT (hEST2), the catalytic subunit of telomerase.

L5 ANSWER 3 OF 8 MEDLINE
TI Interaction of recombinant Tetrahymena telomerase proteins p80 and p95 with telomerase RNA and telomeric DNA substrates.

L5 ANSWER 4 OF 8 MEDLINE
TI Influence of ATM function on telomere metabolism.

L5 ANSWER 5 OF 8 MEDLINE
TI Characterization and cell cycle regulation of the related human telomeric proteins Pin2 and TRF1 suggest a role in mitosis.

L5 ANSWER 6 OF 8 MEDLINE
TI Genomic instability and telomerase activity in human bronchial epithelial cells during immortalization by human papillomavirus-16
E6 and E7 genes.

L5 ANSWER 7 OF 8 MEDLINE
TI Control of telomere length by the human telomeric protein TRF1 [see comments].

L5 ANSWER 8 OF 8 MEDLINE
TI Reconstitution of human telomerase activity and identification of a minimal functional region of the human telomerase RNA.

L5 ANSWER 2 OF 8 MEDLINE
AN 1998187713 MEDLINE DN 98187713
TI Telomerase activity is restored in human cells by ectopic expression of hTERT (hEST2), the catalytic subunit of telomerase.
AU Counter C M; Meyerson M; Eaton E N; Ellisen L W; Caddle S D; Haber D A; Weinberg R A
CS Whitehead Institute for Biomedical Research, Department of Biology, Massachusetts Institute of Technology, Cambridge 02142, USA.
NC CA 39826 (NCI)
SO ONCOGENE, (1998 Mar 5) 16 (9) 1217-22. Journal code: ONC. ISSN: 0950-9232.
CY ENGLAND: United Kingdom DT Journal; Article; (JOURNAL ARTICLE) LA English
FS Priority Journals; Cancer Journals EM 199806 EW 19980604
AB The expression of telomerase, the enzyme that synthesizes telomeric DNA de novo, is suppressed in normal somatic human cells but is reactivated during tumorigenesis. This reactivation appears to arrest the normal loss of telomeric DNA incurred as human cells divide. Since continual loss of telomeric DNA is predicted to eventually limit cell proliferation, activation of telomerase in cancer cells may represent an important step in the acquisition of the cell immortalization which occurs during tumor progression. The telomerase holoenzyme is composed of both RNA and protein subunits. In humans, mRNA expression of hTERT (hEST2), the candidate telomerase catalytic subunit gene, appears to parallel the levels of telomerase enzyme activity, suggesting that induction of hTERT is necessary and perhaps sufficient for expression of telomerase activity in tumor cells. To test this model directly, we ectopically expressed an epitope-tagged version of hTERT in telomerase-negative cells and show that telomerase activity was induced to levels comparable to those seen in immortal telomerase-positive cells and that the expressed hTERT protein was physically associated with the cellular telomerase activity. We conclude that synthesis of the hTERT telomerase subunit represents the rate-limiting determinant of telomerase activity in these cells and that this protein, once expressed, becomes part of the functional telomerase holoenzyme.
CT Check Tags: Human; Support, Non-U.S. Gov't; Support, U.S. Gov't, P.H.S.
Cell Line
HL-60 Cells
Macromolecular Systems
*Proteins: BI, biosynthesis
Proteins: ME, metabolism
* Recombinant Proteins: BI, biosynthesis*
* Recombinant Proteins: ME, metabolism*
* Telomerase: BI, biosynthesis*
Telomerase: ME, metabolism
Transfection
Tumor Cells, Cultured
CN EC 2.7.7.- (Telomerase); 0 (Est2 protein); 0 (Macromolecular Systems); 0 (Proteins); 0 (Recombinant Proteins)

L6 ANSWER 1 OF 9 MEDLINE
TI Telomerase activity in human development is regulated by human telomerase reverse transcriptase (*hTERT*) transcription and by alternate splicing of *hTERT* transcripts. PY 1998

L6 ANSWER 2 OF 9 MEDLINE
TI Telomerase activity and expression of telomerase RNA component and telomerase catalytic subunit gene in cervical cancer. PY 1998

L6 ANSWER 3 OF 9 MEDLINE
TI Telomerase activity exclusively in cervical carcinomas and a subset of cervical intraepithelial neoplasia grade III lesions: strong association with elevated messenger RNA levels of its catalytic subunit and high-risk human papillomavirus DNA. PY 1998

L6 ANSWER 4 OF 9 MEDLINE
TI Expression of human telomerase subunits and correlation with telomerase activity in urothelial cancer. PY 1998

L6 ANSWER 5 OF 9 MEDLINE
TI Structure and regulation mechanisms of telomerase. PY 1998

L6 ANSWER 6 OF 9 MEDLINE
TI Expression of mouse telomerase reverse transcriptase during development, differentiation and proliferation. PY 1998

L6 ANSWER 7 OF 9 MEDLINE
TI Telomerase activity is restored in human cells by ectopic expression of *hTERT* (hEST2), the catalytic subunit of telomerase. PY 1998

L6 ANSWER 8 OF 9 MEDLINE
TI Telomeres and senescence: the history, the experiment, the future. PY 1998

L6 ANSWER 9 OF 9 MEDLINE
TI Reconstitution of telomerase activity in normal human cells leads to elongation of telomeres and extended replicative life span. PY 1998

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(FILE 'USPAT' ENTERED AT 15:26:30 ON 23 DEC 1998)
L1 47 S TELOMERASE
L2 292542 S CATAL? OR ENZYM?
L3 38 S L1(P)L2
L4 195183 S HUMAN OR MOUSE OR MURINE
L5 35 S L1(P)L4
L6 33 S L5 AND L3
L7 1764 S 536/23.5/CCLS
L8 2 S L1 AND L7

L6
1. 5,846,723, Dec. 8, 1998, Methods for detecting the RNA component of telomerase; Nam Woo Kim, et al., 435/6, 91.2, 91.3; 536/23.1, 24.3, 24.33 [IMAGE AVAILABLE]

2. 5,840,495, Nov. 24, 1998, Methods for diagnosis of conditions associated with elevated levels of telomerase activity; Michael D. West, et al., 435/6, 325, 375; 514/44; 536/23.1, 24.1, 24.3, 24.5 [IMAGE AVAILABLE]

3. 5,840,490, Nov. 24, 1998, Telomerase activity associated with hematological and colorectal malignancies; Silvia Bacchetti, et al., 435/6, 5, 91.1, 91.2; 536/24.3, 24.31, 24.32, 24.33 [IMAGE AVAILABLE]

4. 5,837,857, Nov. 17, 1998, Mammalian telomerase; Bryant Villeponteau, et al., 536/24.31; 435/91.31, 196; 536/24.3 [IMAGE AVAILABLE]

5. 5,837,453, Nov. 17, 1998, Telomerase activity assays; Calvin Bruce Harley, et al., 435/6, 91.2, 174; 530/300, 350; 536/23.1, 24.3, 24.33 [IMAGE AVAILABLE]

6. 5,834,193, Nov. 10, 1998, Methods for measuring telomere length; Michael R. Kozlowski, et al., 435/6; 536/24.31 [IMAGE AVAILABLE]

7. 5,830,644, Nov. 3, 1998, Method for screening for agents which increase telomerase activity in a cell; Michael D. West, et al., 435/6, 4, 7.2, 15, 91.2; 436/34, 63, 64, 94, 501 [IMAGE AVAILABLE]

8. 5,804,443, Sep. 8, 1998, Human monocytic leukemia cell line; Carl Bernofsky, 435/372.1, 372 [IMAGE AVAILABLE]

9. 5,804,380, Sep. 8, 1998, Telomerase activity assays; Calvin Bruce Harley, et al., 435/6, 91.2, 91.5, 91.52, 183, 184, 194, 810; 536/24.31, 24.33 [IMAGE AVAILABLE]

10. 5,776,679, Jul. 7, 1998, Assays for the DNA component of "human" "telomerase"; Bryant Villeponteau, et al., 435/6, 91.2, 91.21, 91.51; 536/23.1, 24.31, 24.33 [IMAGE AVAILABLE]

11. 5,770,613, Jun. 23, 1998, Telomerase inhibitors; Federico C. A. Gaeta, et al., 514/332, 335, 336, 337, 340, 342, 344, 345, 350, 352; 546/261, 288, 298, 301, 310, 312 [IMAGE AVAILABLE]

12. 5,770,422, Jun. 23, 1998, "Human" "telomerase"; Kathleen Collins, 435/194, 252.3, 320.1; 530/350, 412; 536/23.2 [IMAGE AVAILABLE]

13. 5,767,278, Jun. 16, 1998, Telomerase inhibitors; Federico C.A. Gaeta, et al., 546/261; 514/335, 344; 546/288 [IMAGE AVAILABLE]

14. 5,760,062, Jun. 2, 1998, Telomerase inhibitors; Federico C.A. Gaeta, et al., 514/344; 546/288, 291, 297, 298 [IMAGE AVAILABLE]

15. 5,747,317, May 5, 1998, "Human" "telomerase" RNA interacting protein gene; Zhaodan Cao, 435/194, 91.31, 252.3, 320.1; 530/350; 536/23.1, 23.2, 24.31 [IMAGE AVAILABLE]

16. 5,741,677, Apr. 21, 1998, Methods for measuring telomere length; Michael R. Kozlowski, et al., 435/91.2, 6, 174; 536/23.1, 24.3, 24.33 [IMAGE AVAILABLE]

17. 5,733,730, Mar. 31, 1998, Telomere repeat binding factor and diagnostic and therapeutic use thereof; Titia De Lange, 435/6, 7.1 [IMAGE AVAILABLE]

18. 5,707,795, Jan. 13, 1998, Therapy and diagnosis of conditions related to telomere length and/or telomerase activity; Michael D. West, et al., 435/5, 4, 6, 91.2; 436/63, 64; 536/24.33 [IMAGE AVAILABLE]

19. 5,703,116, Dec. 30, 1997, Telomerase Inhibitors; Federico C. A. Gaeta, et al., 514/443; 549/57 [IMAGE AVAILABLE]

20. 5,698,686, Dec. 16, 1997, Yeast telomerase compositions; Daniel E. Gottschling, et al., 536/23.1; 435/6, 91.2; 536/22.1, 24.3, 24.31, 24.33 [IMAGE AVAILABLE]

21. 5,695,932, Dec. 9, 1997, Telomerase activity assays for diagnosing pathogenic infections; Michael D. West, et al., 435/6, 91.1 [IMAGE AVAILABLE]

22. 5,693,474, Dec. 2, 1997, Methods for cancer diagnosis and prognosis; Jerry Shay, et al., 435/6, 15, 91.2, 183, 184, 194; 436/63, 64 [IMAGE AVAILABLE]

23. 5,686,306, Nov. 11, 1997, Methods and reagents for lengthening telomeres; Michael D. West, et al., 435/346, 6, 375; 536/23.1 [IMAGE AVAILABLE]

24. 5,686,245, Nov. 11, 1997, Methods for screening for agents which modulate telomere length; Michael D. West, et al., 435/6, 4, 15, 91.1, 91.2; 436/64; 514/44 [IMAGE AVAILABLE]

25. 5,656,638, Aug. 12, 1997, Telomerase inhibitors; Federico C. A. Gaeta, et al., 514/301; 546/114 [IMAGE AVAILABLE]

26. 5,648,215, Jul. 15, 1997, Telomerase diagnostic methods; Michael D. West, et al., 435/6, 15, 91.1, 91.5; 436/64 [IMAGE AVAILABLE]

27. 5,645,986, Jul. 8, 1997, Therapy and diagnosis of conditions related to telomere length and/or telomerase activity; Michael D. West, et al., 435/6, 91.2, 183, 184, 194; 436/63; 536/24.31, 24.33 [IMAGE AVAILABLE]

28. 5,643,890, Jul. 1, 1997, Synthetic oligonucleotides which mimic telomeric sequences for use in treatment of cancer and other diseases; Patrick L. Iversen, et al., 514/44; 424/93.21; 435/375; 536/23.1 [IMAGE AVAILABLE]

29. 5,639,613, Jun. 17, 1997, Methods for cancer diagnosis and prognosis; Jerry Shay, et al., 435/6, 91.2, 183, 184, 194; 436/63, 64 [IMAGE AVAILABLE]

30. 5,629,154, May 13, 1997, Telomerase activity assays; Nam W. Kim, et al., 435/6; 424/94.1; 435/91.2, 174; 536/23.1, 24.3, 24.33 [IMAGE AVAILABLE]

31. 5,583,016, Dec. 10, 1996, Mammalian telomerase; Bryant Villeponteau, et al., 435/91.3, 91.1, 91.31, 194, 252.3, 254.11, 320.1, 366, 369; 536/23.1, 23.2, 24.31, 24.33 [IMAGE AVAILABLE]

32. 5,489,508, Feb. 6, 1996, Therapy and diagnosis of conditions related to telomere length and/or telomerase activity; Michael D. West, et al., 435/6, 15, 91.1, 91.5; 436/64; 536/24.33 [IMAGE AVAILABLE]

33. 5,466,576, Nov. 14, 1995, Modulation of PIF-1-type helicases; Vincent P. Schulz, et al., 435/6, 183, 193 [IMAGE AVAILABLE]

US PAT NO: 5,837,857 [IMAGE AVAILABLE] L6: 4 of 33
BSUM(3) The present invention relates to "human" "telomerase", a ribonucleoprotein "enzyme" involved in "human" telomere DNA synthesis. The invention provides methods and compositions relating to the fields of molecular biology, chemistry, pharmacology, and medical . .

BSUM(5) The DNA at the ends or telomeres of the chromosomes of eukaryotes usually consists of tandemly repeated simple sequences. "Telomerase" is a ribonucleoprotein "enzyme" that synthesizes one strand of the telomeric DNA using as a template a sequence contained within the RNA component of the "enzyme". See Blackburn, 1992, Annu. Rev. Biochem. 61:113-129, incorporated herein by reference.

BSUM(6) The RNA component of "human" "telomerase" has not been reported in the scientific literature to date, although "human" "telomerase" is known to synthesize telomeric repeat units with the sequence 5'-TTAGGG-3'. See Morin, 1989, Cell 59:521-529, and Morin, 1991, Nature. . . been sufficient to enable the isolation and identification of the remainder of the nucleotide sequence of the RNA component of "human" "telomerase". The RNA component of the "telomerase" "enzymes" of Saccharomyces cerevisiae, certain species of Tetrahymena, as well as that of other ciliates, such as Euplotes and Glaucoma, has. . . Blackburn, 1991, Cell 67:343-353; and Shippen-Lentz and Blackburn, 1990, Science 247:546-552, each of w hich is incorporated herein by reference. The "telomerase" "enzymes" of these ciliates synthesize telomeric repeat units distinct from that in humans.

BSUM(7) There is a great need for more information about "human" "telomerase". Despite the seemingly simple nature of the repeat units of telomeric DNA, scientists have long known that telomeres have an. . . speculated that loss of telomeric DNA may act as a trigger of cellular senescence and aging and that regulation of "telomerase" may have important biological implications. See Harley, 1991, Mutation Research 256:271-282, incorporated herein by reference.

BSUM(11) In . . . first aspect, the present invention provides the RNA component of, as well as the gene for the RNA component of, "human" "telomerase" in substantially pure form, as well as nucleic acids comprising all or at least a useful portion of the nucleotide sequence of the RNA component of "human" "telomerase". The present invention also provides RNA component nucleic acids from other species, which nucleic acids share substantial homology with the RNA component of "human" "telomerase", including but not limited to, the RNA components of mammals, such as primates. Other useful nucleic acids of the invention. . . component and which interact with the RNA component or the gene for the RNA component or the protein components of "human" "telomerase" in a useful way; and nucleic acids that do not share significant sequence homology or complementarity to the RNA component. . .

BSUM(12) Thus, . . . triple helix-forming oligonucleotide, or other oligonucleotide that can be used in vivo or in vitro to inhibit the activity of "human" "telomerase". Such oligonucleotides can block "telomerase" activity in a number of ways, including by preventing transcription of the "telomerase" gene (for instance, by triple helix formation) or by binding to the RNA component of "telomerase" in a manner that prevents a functional ribonucleoprotein "telomerase" from assembling or prevents the RNA component, once assembled into the "telomerase" "enzyme" complex, from serving as a template for telomeric DNA synthesis. Typically, and depending on mode of action, these oligonucleotides of. . . or more nucleotides that is either identical or complementary to a specific sequence of nucleotides in the RNA component of "telomerase" or the gene for the RNA component of "telomerase".

BSUM(13) Another type of useful nucleic acid of the invention is a ribozyme able to cleave specifically the RNA component of "human" "telomerase", rendering the "enzyme" inactive. Yet another type of useful nucleic acid of the invention is a probe or primer that binds specifically to the RNA component of "human" "telomerase" and so can be used, e.g., to detect the presence of "telomerase" in a sample. Finally, useful nucleic acids of the invention include recombinant expression plasmids for producing the nucleic acids of the invention. One especially useful type of such a plasmid is a plasmid used for "human" gene therapy. Useful plasmids of the invention for "human" gene therapy come in a variety of types, including not only those that encode antisense oligonucleotides or ribozymes but also those that drive expression of the RNA component of "human" "telomerase" or a deleted or otherwise altered (mutated) version of the RNA component of "human" (or other species with RNA component sequences substantially homologous to the "human" RNA component) "telomerase" or the gene for the same.

BSUM(14) In a second aspect, the invention provides methods for treating a condition associated with the "telomerase" activity within a cell or group of cells by contacting the cell(s) with a therapeutically effective amount of an agent that alters "telomerase" activity in that cell. Such agents include the "telomerase" RNA component-encoding nucleic acids, triple helix-forming oligonucleotides, antisense oligonucleotides, ribozymes, and plasmids for "human" gene therapy described above. In a related aspect, the invention provides pharmaceutical compositions comprising these therapeutic agents together with a. . .

BSUM(15) In a third aspect, the invention provides diagnostic methods for determining the level, amount, or presence of the RNA component of "human" "telomerase", "telomerase", or "telomerase" activity in a cell, cell population, or tissue sample, or an extract of any of the foregoing. In a related. . .

BSUM(16) In a fourth aspect, the present invention provides recombinant "telomerase" preparations and methods for producing such preparations. Thus, the present invention provides a recombinant "human" "telomerase" that comprises the protein components of "human" "telomerase" as well as the protein components of "telomerase" from a mammalian species with an RNA component substantially homologous to the RNA component of "human" "telomerase" in association with a recombinant RNA component of the invention. Such recombinant RNA component molecules of the invention include those. . . to a naturally occurring RNA component molecule that are produced in recombinant host cells. The method for producing such recombinant "telomerase" molecules comprises transforming a eukaryotic host cell that expresses the protein components of "telomerase" with a recombinant expression vector that encodes an RNA component molecule of the invention, and culturing said host cells transformed. . . said vector under conditions such that the protein components and RNA component are expressed and assemble to form an active "telomerase" molecule capable of adding sequences (not necessarily the same sequence added by native "telomerase") to telomeres of chromosomal D[redacted]

BSUM(17) In a fifth aspect, the invention provides methods for purifying the protein components of "human" "telomerase" as well as the protein components of "telomerase" from a mammalian species with an RNA component substantially homologous to the RNA component of "human" "telomerase". The present invention also provides methods for isolating and identifying nucleic acids encoding such protein components. In related aspects, the present invention provides purified "human" "telomerase" and purified "telomerase" of mammalian species with an RNA component substantially homologous to the RNA component of "human" "telomerase", as well as purified nucleic acids that encode one or more components of such "telomerase" preparations. The present invention also provides pharmaceutical compositions comprising as an active ingredient the protein components of "telomerase" or a nucleic acid that encodes or interacts with a nucleic acid that encodes a protein component of "telomerase".

BSUM(20) The present invention provides methods, reagents, and pharmaceutical compositions relating to the ribonucleoprotein "human" "telomerase". The invention in part arises out of the cloning and isolation of the RNA component of "human" "telomerase" and the gene for that RNA component. The nucleotide sequence of the RNA component of "human" "telomerase" is shown below. For convenience, the sequence is shown using the standard abbreviations for ribonucleotides (A is riboadenine, G is. . .

BSUM(22) The cloning of the RNA component of "human" "telomerase" required a novel method involving negative selection and cycles of positive selection, described below. Initially, however, an attempt was made. . . amplification. The reverse transcription reaction was initiated with a primer identical to the repeat unit in the single-strand portion of "human" telomeric DNA and thus c[redacted]entary to a sequence believed to be present in the RNA component of "human" "te[redacted]rase". The primer also comprised, at its 5'-end, a sequence corresponding to a restriction "enzyme" recognition site. However, when the cDNA produced by the reverse transcription reaction and PCR amplification was examined by gel electrophoresis. . .

BSUM(23) The successful cloning effort began with the preparation of cDNA from purified preparations of "human" "telomerase" as well as from cell lines that have "human" "telomerase" activity and from cell lines that do not have detectable "human" "telomerase" activity. The method used to prepare the cDNA is described in detail in Example 1, below. Two negative selection steps and successive cycles of positive selection were used in conjunction with the cDNA preparations from the two "human" cell lines to lower the concentration of unwanted sequences and to raise the concentration of the desired RNA component sequences.

BSUM(24) The negative selection steps involved the preparation of biotinylated PCR product from cDNA prepared from a "human" cell line that does not have detectable "telomerase" activity. The biotinylated PCR product was denatured and then rehybridized in a solution comprising a much lower concentration of non-biotinylated PCR product (100 biotinylated product:1 non-biotinylated product) from cDNA prepared from a "human" cell line that does have "telomerase" activity. Given the possibility that the "telomerase" negative cell line might contain some low amount of the RNA component, the hybridization step was conducted to discriminate or. . . binding to streptavidinylated magnetic particles; the supernatant remaining after particle collection contained the desired cDNA for the RNA component of "human" "telomerase". The process for PCR amplification of cDNA is described in Example 2, below.

BSUM(25) This . . . selection. In the positive selection step, a biotinylated probe complementary to the predicted template sequence in the RNA component of "human" "telomerase" was hybridized to PCR product from an enriched (by negative selection) sample of the PCR-amplified cDNA from a "human" cell line that has "telomerase" activity. After hybridization, the probe/target complexes were bound to avidinylated magnetic beads, which were then collected and used as a. . .

BSUM(26) After . . . acids were then eluted from the gel sections and amplified by PCR. The PCR amplification products were digested with restriction "enzyme" NotI and then inserted by ligation into the NotI site of plasmid pBluescriptIISK+, commercially available from Stratagene. The resulting plasmids. . . were prepared and hybridized to a probe comprising a telomeric repeat sequence and therefore complementary to the RNA component of "human" "telomerase". A number of clones positive by this test were then analyzed by DNA sequencing and a variety of other tests.

BSUM(27) These other tests included the following: (1) determination whether antisense oligonucleotides complementary to the putative RNA component would inhibit "telomerase" activity in "human" cell extracts known to contain "telomerase"; (2) determination whether PCR primers specific for a putative RNA component clone sequence could be used to amplify a nucleic acid present in a "telomerase" sample and whether the product observed, if any, would track "telomerase" activity during purification of "telomerase"; and (3) determination whether PCR primers specific for a putative RNA component clone sequence could be used to amplify a nucleic acid present in greater abundance in cell extracts from cells in which "telomerase" activity is known to be high (i.e., tumor cells) than in cell extracts from cells known to produce no or only low amounts of "telomerase" activity. One clone, designated plasmid pGRN7, produced results in these tests consistent with the determination that the plasmid comprised cDNA corresponding to the RNA component of "human" "telomerase".

BSUM(29) The above results provided convincing evidence that the RNA component of "human" "telomerase" had been cloned, so plasmid pGRN7 was then used to isolate a genomic clone for the RNA component from a "human" cell line, as described in Example 7, below. The genomic clone was identified in and isolated from a genomic library of "human" DNA inserted into a lambda vector FIXII purchased from Stratagene. The desired clone comprising the RNA component gene sequences contained. . . localized to the distal end of the q arm of chromosome 3. The sequence information obtained from a SauIIIA1 restriction "enzyme" recognition site at one end of the .about.15kb insert to an internal HindIII restriction "enzyme" recognition site, which comprises all of the mature RNA component sequence as well as transcription control elements of the RNA. . .

BSUM(30) The plasmids described above that were constructed during the cloning of the RNA component of "human" "telomerase" and the gene for the RNA component are important aspects of the present invention. These plasmids can be used to produce the RNA component of, as well as the gene for, "human" "telomerase" in substantially pure form, yet another important aspect of the present invention. In addition, those of skill in the art. . . pure form, that comprise all or at least a useful portion of the nucleotide sequence of the RNA component of "human" "telomerase" are useful materials provided by the present invention.

BSUM(32) Just . . . the invention is an antisense oligonucleotide that can be used in vivo or in vitro to inhibit the activity of "human" "telomerase". Antisense oligonucleotides comprise a specific sequence of from about 10 to about 25 to 200 or more (i.e., large enough. . . delivery, to administer in vivo, if desired) nucleotides complementary to a specific sequence of nucleotides in the RNA component of "human" "telomerase". The mechanism of action of such oligonucleotides can involve binding of the RNA component either to prevent assembly of the functional ribonucleoprotein "telomerase" or to prevent the RNA component from serving as a template for telomeric DNA synthesis.

BSUM(33) Illustrative antisense oligonucleotides of the invention that serve to inhibit "telomerase" activity in vivo and/or in vitro include the oligonucleotides mentioned above in connection with the tests to determine whether clone pGRN7 comprised the cDNA for the RNA component of "human" "telomerase". Three such oligonucleotides were synthesized as 2'-O-methyl RNA oligonucleotides, which bind more tightly to RNA than DNA oligonucleotides and are more resistant to hydrolysis than unmodified RNA oligonucleotides, and, as noted above, were used to demonstrate inhibition of "telomerase" activity in vitro. The sequence of each of these O-methyl RNA oligonucleotides is shown below.

BSUM(37)These oligonucleotides can also be used to inhibit "telomerase" activity in "human" cells.

BSUM(40) In . . . helix-forming oligonucleotides of the invention, "sense" oligonucleotides identical in sequence to at least a portion of the RNA component of "human" "telomerase" can also be used to inhibit "telomerase" activity. Oligonucleotides of the invention of this type are characterized in comprising either (1) less than the complete sequence of the RNA component needed to form a functional "telomerase" "enzyme" or (2) the complete sequence of the RNA component needed to form a functional "telomerase" "enzyme" as well as a substitution or insertion of one or more nucleotides that render the resulting RNA non-functional. In both cases, inhibition of "telomerase" activity is observed due to the "mutant" RNA component binding the protein components of "human" "telomerase" to form an inactive "telomerase" molecule. The mechanism of action of such oligonucleotides thus involves the assembly of a non-functional ribonucleoprotein "telomerase" or the prevention of assembly of a functional ribonucleoprotein "telomerase". Sense oligonucleotides of the invention of this type typically comprise a specific sequence of from about 20, 50 200, 400, 500, or more nucleotides identical to a specific sequence of nucleotides in the RNA component of "human" "telomerase".

BSUM(41) Thus, another useful oligonucleotide of the invention comprises an altered or mutated sequence of the RNA component of "human" "telomerase". Yu et al., 1990, Nature 344: 126, shows that a mutated form of the RNA component of Tetrahymena "telomerase" can be incorporated into the "telomerase" of Tetrahymena cells and that the incorporation has deleterious effects on those cells. Incorporation of mutated forms of the RNA component of "human" "telomerase" may have similar effects on "human" cells that otherwise have "telomerase" activity without affecting normal "human" cells that do not have "telomerase" activity. Such mutated forms include those in which the sequence 5'-CTAACCCTA-3'[SEQ ID NO:8] is mutated to 5'-CAAACCCAA-3', [SEQ ID NO:9]. . . units incorporated into the chromosomal DNA, thus affecting chromosome structure and function. Such oligonucleotides can be designed to contain restriction "enzyme" recognition sites useful in diagnostic methods for the presence of the altered RNA component via restriction "enzyme" digestion of telomeric DNA or an extended "telomerase" substrate.

BSUM(43) The assays showed that the "telomerase" activity in the cells resulted in the formation of nucleic acids comprising the altered sequences, indicating that the genomic clone. . . that the plasmids comprised an altered but functional RNA component gene. These results illustrate how the present invention provides recombinant "telomerase" preparations and methods for producing such preparations. The present invention provides a recombinant "human" "telomerase" that comprises the protein components of "human" "telomerase" in functional association with a recombinant RNA component of the invention. Such recombinant RNA component molecules of the invention include. . . to a naturally occurring RNA component molecule that are produced in recombinant host cells. The method for producing such recombinant "telomerase" molecules comprises transforming a eukaryotic host cell that expresses the protein components of "telomerase" with a recombinant expression vector that encodes an RNA component molecule of the invention, and culturing said host cells transformed. . . said vector under conditions such that the protein components and RNA component are expressed and assemble to form an active "telomerase" molecule capable of adding sequences (not necessarily the same sequence added by native "telomerase") to telomeres of chromosomal DNA. Other useful embodiments of such recombinant DNA expression vectors (or plasmids) include plasmids that comprise the gene for the RNA component of "human" "telomerase" with a deletion, insertion, or other modification that renders the gene non-functional. Such plasmids are especially useful for "human" gene therapy to "knock-out" the endogenous RNA component gene, although a highly efficient transformation and recombination system is required, to. . .

BSUM(44) Other oligonucleotides of the invention called "ribozymes" can also be used to inhibit "telomerase" activity. Unlike the antisense and other oligonucleotides described above, which bind to an RNA, a DNA, or a "telomerase" protein component, a ribozyme not only binds but also specifically cleaves and thereby potentially inactivates a target RNA, such as the RNA component of "human" "telomerase". Such a ribozyme can comprise 5'- and 3'-terminal sequences complementary to the "telomerase" RNA. Depending on the site of cleavage, a ribozyme can render the "telomerase" "enzyme" inactive. See PCT patent publication No. 93/23572, supra. Those in the art upon review of the RNA sequence of the "human" "telomerase" RNA component will note that several useful ribozyme target sites are present and susceptible to cleavage by, fo r example, a. . .

BSUM(53) Other therapeutic methods of the invention employ the "telomerase" RNA nucleic acid of the invention to stimulate "telomerase" activity and to extend replicative cell life span. These methods can be carried out by delivering to a cell a functional recombinant "telomerase" ribonucleoprotein of the invention to the cell. For instance, the ribonucleoprotein can be delivered to a cell in a liposome, or the gene for the RNA component of "human" "telomerase" (or a recombinant gene with different regulatory elements) can be used in a eukaryotic expression plasmid (with or without sequences coding for the expression of the protein components of "telomerase") to activate "telomerase" activity in various normal "human" cells that otherwise lack detectable "telomerase" activity due to low levels of expression of the RNA component or a protein component of "telomerase". If the "telomerase" RNA component is not sufficient to stimulate "telomerase" activity, then the RNA component can be transfected along with genes expressing the protein components of "telomerase" to stimulate "telomerase" activity. Thus, the invention provides methods for treating a condition associated with the "telomerase" activity within a cell or group of cells by contacting the cell(s) with a therapeutically effective amount of an agent that alters "telomerase" activity in that cell.

BSUM(59) In related aspects, the invention features pharmaceutical compositions including a therapeutically effective amount of a "telomerase" inhibitor or "telomerase" activator of the invention. Pharmaceutical compositions of "telomerase" inhibitors of the invention include a mutant RNA component of "human" "telomerase", an antisense oligonucleotide or triple helix-forming oligonucleotide that binds the RNA component or the gene for the same of "human" "telomerase", or a ribozyme able to cleave the RNA component of "human" "telomerase", or combinations of the same or other pharmaceuticals in a pharmaceutically acceptable carrier or salt. Other pharmaceutical compositions of the invention comprise a "telomerase" activator preparation, such as purified "human" "telomerase" or mRNA for the protein components of "telomerase" and the RNA component of "telomerase", and are used to treat senescence-related disease. The therapeutic agent can be provided in a formulation suitable for parenteral, nasal,. .

BSUM(60) The . . . methods described above. The invention provides diagnostic methods for determining the level, amount, or presence of the RNA component of "human" "telomerase", "telomerase", or "telomerase"activity in a cell, cell population, or tissue sample. In a related aspect, the present invention provides useful reagents for. . . above in connection with the tests conducted to determine that clone pGRN7 contained the cDNA for the RNA component of "human" "telomerase", the levels of the RNA component are elevated in tumor cells. Thus, detection of the RNA component is a useful. . .

1) In addition, probes or primers that bind specifically to the RNA component of "human" ase" (or either strand of the gene for the same) can be used in diagnostic methods to detect the presence of "telomerase" nucleic acid in a sample. Primers and probes are oligonucleotides that are complementary, and so will bind, to a target. . .

BSUM(63) Depending . . . the length and intended function of the primer, probe, or other nucleic acid comprising sequences from the RNA component of "human" "telomerase", expression plasmids of the invention may be useful. For instance, recombinant production of the full-length RNA component of the invention. . .

BSUM(64) The . . . in the 5'-region of the gene, and RNA component coding region, can be used to express the RNA component in "human" cells, including "human" cells that have been immortalized by viral transformation or cancer. The promoter of the RNA component gene may be regulated,. . . component gene to a coding sequence for a "reporter" coding sequence, such as the coding sequence for beta-galactosidase or another "enzyme" or protein the expression of which can be readily monitored. Thus, the promoter and other regulatory elements of the gene for the RNA component of "human" "telomerase" can be used not only to express the RNA component but also protein components of "human" "telomerase", antisense or other oligonucleotides, as well as other gene products of interest in "human" cells. Expression plasmids comprising the intact gene for the RNA component of "human" "telomerase" can be especially useful for a variety of purposes, including gene therapy. Those of skill in the art recognize that. . .

BSUM(65) As indicated by the foregoing description, access to purified nucleic acids comprising the sequence of the RNA component of "human" "telomerase" provides valuable diagnostic and th[redacted]ic methods and reagents, as well as other important benefits. One important benefit of th[redacted]ent. . . and reagents of the invention can be used to isolate the RNA component and genes for the RNA component of "telomerase" from any mammalian species that has an RNA component substantially homologous to the "human" RNA component of the present invention. The phrase "substantially homologous" refers to that degree of homology required for specific hybridization of an oligonucleotide or nucleic acid sequence of the "human" RNA component to a nucleic acid sequence of an RNA component sequence of another mammalian species. Given such substantial homology,. . .

BSUM(66) For . . . these and other similar techniques, those of ordinary skill can readily isolate not only variant RNA component nucleic acids from "human" cells but also homologous RNA component nucleic acids from other mammalian cells, such as cells from primates, from mammals of. . . nucleic acids can be used to prepare transgenic animals of great value for screening and testing of pharmaceuticals that regulate telomerase" activity. For instance, by using a plasmid of the invention, one can "knock out" the RNA component gene or replace. . . RNA component gene with a recombinant inducible gene in a mus spretus embryonic stem cell and then generate a transgenic "mouse" that will be useful as a model or test system for the study of age- or senescence-related disease. Example 9,. . .

BSUM(67) The reagents of the present invention also allow the cloning and isolation of nucleic acids encoding the protein components of "human" as well as other mammalian "telomerase" "enzymes", which have not previously been available. Access to such nucleic acids provide complementary benefits to those provided by the nucleic acids comprising nucleic acid sequences of the RNA component of "human" "telomerase". For instance, and as noted above, the therapeutic benefits of the present invention can be enhanced, in some instances, by use of purified preparations of the protein components of "human" "telomerase" and by access to nucleic acids encoding the same. The nucleic acids of the invention that encode the RNA component of "human" "telomerase" can be used to isolate the nucleic acid encoding the protein components of "human" "telomerase", allowing access to such benefits. Thus, the invention provides methods for isolating and purifying the protein components of "human" "telomerase", as well as for identifying and isolating nucleic acids encoding the protein components of "human" "telomerase". In related aspects, the present invention provides purified "human" "telomerase", purified nucleic acids that encode the protein components of "human" "telomerase", recombinant expression plasmids for the protein components of "human" "telomerase". The invention also provides pharmaceutical compositions comprising as an active ingredient either the protein components of "human" "telomerase" or a nucleic acid that either encodes those protein components or interacts with nucleic acids that encode those protein components,. . .

BSUM(68) The cloned RNA component of "human" "telomerase" can be used to identify and clone nucleic acids encoding the protein components of the ribonucleoprotein "telomerase" "enzyme". Several different methods can be employed to achieve identification and cloning of the protein components. For instance, one can use affinity capture of the "enzyme" or partially denatured "enzyme" using as an affinity ligand either (1) nucleotide sequences complementary to the RNA component to bind to the RNA component of the intact "enzyme"; or (2) the RNA component to bind the protein components of a partially or fully denatured "enzyme". The ligand can be affixed to a solid support or chemically modified (e.g., biotinyiated) for subsequent immobilization on the support. Exposure of cell extracts containing "human" "telomerase", followed by washing and elution of the "telomerase" "enzyme" bound to the support, provides a highly purified preparation of the "telomerase" "enzyme". The protein components can then be optionally purified further or directly analyzed by protein sequencing. The protein sequence determined can. . . cloning the cDNA or identifying a clone in a genomic bank comprising nucleic acids that encode a protein component of "telomerase".

BSUM(69) Affinity capture of "telomerase" utilizing an engineered RNA component can also be conducted using in vitro transcribed "telomerase" RNA and a system for the reconstitution of "telomerase" "enzyme" activity. See Autexier and Greider, 1994, Genes & Development 8:563-575, incorporated herein by reference. The RNA is engineered to contain. . . sequence-specific nucleic acid binding protein, or an organic dye that binds tightly to a specific RNA sequence. The tolerance of "telomerase" for the tag sequence and position can be tested using standard methods. Synthesis of the altered RNA component and the. . . out in vivo. Affinity capture using the immobilized ligand for the RNA tag can then be used to isolate the "enzyme".

BSUM(70) Expression screening can also be used to isolate the protein components of the "telomerase" "enzyme". In this method, cDNA expression libraries can be screened with labeled "telomerase" RNA, and cDNAs encoding proteins that bind specifically to "telomerase" RNA can be identified. A molecular genetic approach using translational inhibition can also be used to isolate nucleic acids encoding the protein components of the "telomerase" "enzyme". In this method, "telomerase" RNA sequences will be fused upstream of a selectable marker. When expressed in a suitable system, the selectable marker will be functional. When cDNA encoding a "telomerase" RNA binding protein is expressed, the protein will bind to its recognition sequence thereby blocking translation of the selectable marker,. . .

BSUM(71) "Telomerase" RNA binding or "telomerase" activity assays for detection of specific binding proteins and activity can be used to facilitate the purification of the "telomerase" "enzyme" and the identification of nucleic acids that encode the protein components of the "enzyme". For example, nucleic acids comprising RNA component sequences can be used as affinity reagents to isolate, identify, and purify peptides,. . . or other compounds that bind specifically to a sequence contained within the RNA component, such as the protein components of "human" "telomerase". Several different formats are available, including gel shift, filter binding, footprinting, Northwestern (RNA probe of protein blot), and photocrosslinking, to. . .

BSUM(72) As . . . to those of skill in the art upon reading of this disclosure, the present invention provides valuable reagents relating to "human" "telomerase", as well as a variety of useful therapeutic and diagnostic methods. The above description of necessity provides a limited sample. . .

BSUM(73) The . . . to illustrate the invention and provide a description of the methods used to isolate and identify the RNA component of "human" "telomerase" for those of skill in the art. The examples should not be construed as limiting the invention, as the examples. . .

DETD(20) For the positive selection step of the cyclic selection process used to clone the RNA component of "human" "telomerase", about 2 μg of the PCR-amplified cDNA were diluted into 25 μl of TE buffer and then mixed with 1.25. . .

DETD(23)The . . . cyclic selection process is functioning properly with respect to the molecule of interest, in this case the RNA component of "human" "telomerase".

DETD(40) Cloning the gene for the RNA component of "human" "telomerase"

DETD(41) The procedures used to clone the gene for the RNA component of "human" "telomerase" were carried out as generally described in Maniatis et al., Laboratory Molecular Cloning Manual. A genomic DNA library of DNA from the "human" lung fibroblast cell line WI-38 inserted into phage lambda vector FIXII was purchased from Stratagene. The phage were plated at. . .

DETD(43) One strong signal emanated from the filter containing a phage, later designated 28-1, comprising the gene for the RNA component of "human" "telomerase". The plaque corresponding to the signal observed on the filter was used to make secondary plates, so that an isolated. . . comprises several restriction fragments that contain sequences that hybridize with RNA component sequences on pGRN7: a 4.2 kb EcoRI restriction "enzyme" fragment; a 4.2 kb ClaI restriction "enzyme" fragment, and a 2.5 kb HindIII-SacI restriction "enzyme" fragment. The latter fragment comprises the entire .about.560 nucleotide sequence of the RNA component shown above and is believed to comprise the complete gene for the RNA component. The plasmid comprising the 2.5 kb HindIII-SacI restriction "enzyme" fragment in the pBluescript vector was designated plasmid pGRN33 and is available from the American Type Culture Collection under the accession No. ATCC 75926. To the extent the "human" gene may comprise sequences other than those on the 2.5 kb fragment, those sequences can be isolated from phage 28-1. . .

DETD(47) Antisense plasmids for the RNA component of "human" "telomerase"

DETD(51)After . . . plasmid comprises puromycin resistance-conferring, DHFR, and hygromycin B resistance-conferring genes as selectable markers, the SV40 origin of replication; the inducible "human" metallothionein gene promoter positioned for expression of the antisense strand of the gene for the RNA component of "human" "telomerase" (one could also use a stronger promoter to get higher expression levels), and the SV40 late poly A addition site.

DETD(52) The . . . the fibrosarcoma cell line HT1080. HT1080 cells are normally immortal; expression of the antisense RNA for the RNA component of "human" "telomerase" should prevent the RNA component of human" "telomerase" from association with the protein components, blocking the formation of active "telomerase" and rendering the cells mortal.

DETD(56) For . . . from each of these mammalian species can be cloned as described above for the gene for the RNA component of "human" "telomerase".

CLAIMS: We . . . length and that comprises 10 to 500 nucleotides in a sequence identicalor exactly complementary to a contiguous sequence of "human""telomerase" RNA component, the oligonucleotide characterized by theability to specifically hybridize in a cell or tissue sample or extractonly to "human" "telomerase" RNA component or its complement, whereinthe oligonucleotide does not hybridize to telomeric DNA.

4. The oligonucleotide of claim 1 that, when bound to an RNA component of "human" telomeras inhibits or blocks the activity of the "telomerase".

5. . . . plasmid comprising a nucleotide sequence identical or exactly complementary to a contiguous sequence 10 to 500 nucleotides in length of "human" "telomerase" RNA component and further comprising a promoter positioned to drive transcription of an RNA complementary or identical in sequence to the nucleotide sequence, wherein the RNA specifically hybridizes only to "human" "telomerase" RNA component or its complement, and does not hybridize to telomeric DNA.

8. An RNA component of a mammalian "telomerase" in substantially pure form that comprises a sequence that is substantially homologous to a sequence in the RNA component of "human" "telomerase".

9. A method for producing a recombinant "telomerase" "enzyme", said method comprising transforming a eukaryotic host cell that expresses protein components of "telomerase" with a recombinant expression vector that encodes an RNA component of claim 8, and culturing said host cells transformed with. . . said vector under conditions such that the protein components and RNA component are expressed and assemble to form an active "telomerase" molecule capable of adding sequences to telomeres of chromosomal DNA.

10. The oligonucleolide of claim 1 wherein the sequence is identical or exactly complementary to a contiguous sequence contained within a "human" genomic DNA sequence encoding the RNA component of "human""telomerase" located in an .about.2.5 kb HindIII-SacI insert of plasmid pGRN33 (ATCC 75926).

13. . . . oligonucleotide of 10 to 30 nucleotides in length in a sequence identical or exactly complementary to a contiguous sequence of *human* *telomerase* RNA component, wherein the oligonucleotide is hybridized, in a cell or tissue sample or extract, only to a nucleic acid having the sequence of a mammalian *telomerase* RNA component or its complement.

19. The oligonucleotide of claim 13 wherein the mammalian *telomerase* is *human* *telomerase*.

30. . . . plasmid comprising a nucleotide sequence identical or exactly complementary to a contiguous sequence 10 to 500 nucleotides in length of *human* *telomerase* RNA component, and further comprisinga promoter positioned to drive transcription of an RNA complementary or identical in sequence to the nucleotide sequence, wherein the RNA specifically hybridizes only to *human* *telomerase* RNA component or its complement and does not hybridize to telomeric DNA.

33. The host cell of claim 30 wherein the contiguous sequence is contained within a *human* genomic DNA sequence encoding the RNA component of *human* *telomerase* located in an .about.2.5 kb HindIII-SacI insert of plasmid pGRN33 (ATCC 75926).

34. An oligonucleotide probe or primer 10 to 30 nucleotides in length that specifically binds onlv to *human* *telomerase* RNA component or to either strand of a gene encoding *human* *telomerase* RNA component, wherein the oligonucleotide does not hybridize to telomeric DNA.

37. The oligonucleotide of claim 34 that specifically binds to the RNA component of *human* *telomerase*.

38. . . . 34 that is hybridized in a cell or tissue sample or extract to a nucleic acid comprising the sequence of *human* *telomerase* RNA or its complement.

39. The oligonucleotide of claim 34 that specifically binds to a contiguous sequence contained within a *human* genomic DNA sequence encoding *human* *telomerase* RNA component located in an .about.2.5 kb HindIII-SacI insert of plasmid pGRN33 (ATCC 75926).

US PAT NO: 5,770,422 [IMAGE AVAILABLE] L6: 12 of 33
TITLE: *Human* *telomerase*
ABSTRACT: The invention provides methods and compositions rel a *human* *telomerase* and related nucleic acids, including four distinct *human* *telomerase* subunit proteins called p140, p105, p48 and p43 having *human* *telomerase*-specific activity. The proteins may be produced recombinantly from transformed host cells from the disclosed *telomerase* encoding nucleic acids or purified from *human* cells. Also included are *human* *telomerase* RNA components, as well as specific, functional derivatives thereof. The invention provides isolated *telomerase* hybridization probes and primers capable of specifically hybridizing with the disclosed *telomerase* gene, *telomerase*- specific binding agents such as specific antibodies, and ethods of making and using the subject compositions in diagnosis, therapy and in. . .

BSUM(6) DNA . . . occurs during cell replication, in part from incomplete replication of chromosome termini by DNA-dependent DNA polymerase. Telomeric repeat addition is *catalyzed* by the *enzyme* *telomerase*: a ribonucleoprotein *enzyme* which uses a short region within the RNA as a template for the polymerase reaction. Although cells can maintain a constant number of telomeric repeats by balancing repeat loss and addition, not all cells do so. *Human* germline and cancer cells maintain a constant number of telomeric repeats, while normal *human* somatic cells lose telomeric repeats with each cycle of cell division. Cells which do not maintain stable telomere length demonstrate. . .

BSUM(8) Purification of *telomerase* from the ciliate Tetrahymena and cloning of genes encoding two protein components of the *enzyme* is reported in Collins et al. (1995) Cell 81, 677-686 and copending U.S. patent application Ser. No. 08/359,125, filed 19 Dec. 1994. terature relating to *human* *telomerase* include; Kim et al. (1994) Science 266, 2011-2014; and Feng et al. (1995) Science 269, 1236-1241. Literature relating to *telomerase* template modifications include Autexier et al. (1994) Genes and Devel 8, 563-575; Yu et al. (1991) Cell 67, 823-832; and. . .

BSUM(10) The invention provides methods and compositions relating to a *human* *telomerase* and related nucleic acids. Included are four distinct *human* *telomerase* subunit proteins, called p140, p105, p48 and p43 nd *telomerase* protein domains thereof having *telomerase*-specificactivity. The proteins may be produced recombinantly from transformed host cells from the subject *telomerase* encoding nucleic acids or purified from *human* cells. Also included are *human* *telomerase* RNA components, as well as specific, functional derivatives thereof.

BSUM(24) The invention provides isolated *human* *telomerase* proteins including *human* *telomerase* proteins p140, p105, p48 and p43, having molecular weights of about 149 kD, about 105 kD, about 48 kD and. . . kD, respectively, as determined by polyacrylamide gel electrophoresis under denaturing conditions (Matsudaira and Burgess (1978) Anal Biochem 87, 386-396), and *telomerase* protein domains thereof. The *telomerase* proteins comprise assay-discernable functional domains including RNA recognition motifs and subunit binding domains and may be provided as fusion products, e.g with non-*telomerase* polypeptides. The *human* *telomerase* proteins of the invention, including the subject protein domains, all have *telomerase*-specific activity or function.

BSUM(25) *Telomerase*-specific activity or function may be determined by convenient in vitro, cell-based, or in vivo assays: e.g. in vitro binding assays,. . . animals (e.g. immune response, gene therapy, transgenics, etc.), etc. Binding assays encompass any assay where the molecular interaction of a *telomerase* protein with a binding target is evaluated. The binding target may be natural intracellular binding target such as a *telomerase* subunit (e.g. another protein subunit or RNA subunit), a substrate, agonist, antagonist, chaperone, or other regulator that directly modulates *telomerase* activity or its localization; or non-natural binding target such a specific immune protein such as an antibody, or a *telomerase* specific agent such as those identified in assays described below. Generally, *telomerase*-binding specificity is assayed by telomere lymerase activity (see, e.g. Collins et al. 1995, Cell 81, 677-686), by binding equilibrium constants. . . more preferably at least about $10^9 M^{-1}$), by the ability of the subject protein to function as negative mutants in *telomerase*-expressing cells, to elicit *telomerase* specific antibody in a heterologous host (e.g a rodent or rabbit), etc. In any event, the *telomerase* binding specificity of the subject *telomerase* proteins necessarily distinguishes ciliate *telomerase*, preferably distinguishes non-mammalian telomerases and more preferably distinguishes non-*human* telomerases. Exemplary *telomerase* proteins which are shown to have *telomerase* binding specificity include the *telomerase* RNA (e.g. SEQ ID NO:6) binding domains (e.g. RRM 1-4: SEQ ID NO:1, about residues 5-81, residues 115-192, residues 336-420, and residues 487-578, respectively). *telomerase* primer binding domains, nucleotide triphosphate binding domains and binding domains of regulators of *telomerase* such as nuclear localization proteins, etc. As used herein, a protein domain comprises at least 12, preferably at least about. . .

BSUM(26) The claimed *human* *telomerase* proteins are isolated or pure: an isolated" protein is unaccompanied by at least some of the material with which it. . . least about 90%, and eferably at least about 99% by weight of the total protein in a given sample. The *telomerase* proteins and protein domains may be synthesized, produced by recombinant technology, or purified from *human* cells. A wide variety of molecular and biochemical methods are available for biochemical synthesis, molecular expression and purification of the. . . Greene Publ. Assoc., Wiley-Interscience, N.Y.) or that are otherwise known in the art. An exemplary method for isolating each of *human* *telomerase* protein p140, p105, p48 and p43 from *human* cells is as follows:

BSUM(27) Several . . . are supplemented with 15% glycerol and centrifuged at 125,000.times.g for 50 minutes to obtain a first soluble fraction enriched for *telomerase* activity (S-100 fraction). The S-100 fraction is adjusted to 0.2M ammonium sulfate, bound to SP Sepharose (Pharmacia), and developed with a gradient in sodium chloride, to obtain a second soluble fraction enriched for *telomerase* (SP fraction). The SP fraction is adjusted to about 0.3-0.4M ionic strength and bound to Q Sepharose (Pharmacia), and developed with a gradient in sodium chloride, to obtain a third soluble fraction enriched for *telomerase* (Q fraction). The Q fraction is adjusted to about 0.3-0.4M ionic strength, bound to phosphocellulose (Whatman), and developed with sodium chloride, to obtain a fourth soluble fraction enriched for *telomerase* (PC fraction). The PC fraction is adjusted to about 0.3-0.4M ionic strength, bound to 2'Omethyl RNA oligonucleotide immobilized on streptavidin. . . obtain gel protein bands at a molecular weight of about 140 kD, 105 kD, 48 kD or 43 kD having *telomerase* activity. The gel bands are excised or blotted to obtain purified *human* *telomerase* proteins p140, p105, p48 and p43.

BSUM(28) The subject *telomerase* proteins find a wide variety of uses including use in isolating, enriching for and concentrating *telomerase* RNA and *telomerase* proteins, as immunogens, in the methods and applications described below, as reagents in the biotechnology industries, and in therapy. Recombinant. . . molecules. The use of a repeat sequence for 3' end tagging improves specificity and provides sequence alternatives compared with non-templated *enzymes* presently available for this purpose, e.g. terminal transferase. Repeats encoding restriction *enzyme* sites provide repeat tagging to facilitate cloning and the use of *telomerase* alleviates the restrictive conditions required for optimal ligation with available ligase *enzymes*. *Telomerase* also finds use in regulating cell growth or increasing cell density tolerance; for example, cells contacted with an effective amount of exogenous *telomerase* to overcome the growth control limitation otherwise imposed by short telomere length. *Telomerase* may be introduced, expressed, or repressed in specific populations of cells by any convenient way such as microinjection, promoter-specific expression of recombinant *enzyme*, targeted delivery of lipid vesicles, etc. Advantageously, only a brief period of *telomerase* activity is required to allow many generations of continued proliferation of the contacted cell, due to the ability of *telomerase* to extend telo eres in one cell cycle by more sequence than is lost with each cell division.

BSUM(29) The invention provides natural and non-natural *human* *telomerase*-specific binding agents including substrates, agonist, antagonist, etc., methods of identifying and making such agents, and their use in diagnosis, therapy and pharmaceutical development. For example, *human* *telomerase*-specific agents are useful in a variety of diagnostic and therapeutic applications. Novel *human* *telomerase*-specific binding agents include *human* *telomerase*-specific receptors, such as somatically recombined protein receptors like specific antibodies or T-cell antigen receptors (see, e.g Harlow and Lane (1988). . . non-natural intracellular binding agents identified in screens of chemical libraries such as described below, etc. Agents of particular interest modulate *human* *telomerase* function, e.g. *human* *telomerase* antagonists and find use methods for modulating the binding of a *human* *telomerase* or *telomerase* protein to a *human* *telomerase* binding target.

BSUM(31) The amino acid sequences of the disclosed *telomerase* proteins are used to back-translate *telomerase* protein-encoding nucleic acids optimized for selected expression systems (Holler et al. (1993) Gene 136, 323-328; Martin et al. (1995) Gene 154, 150-166) or used to generate degenerate oligonucleotide primers and probes for use in the isolation of natural *telomerase* encoding nucleic acid sequences ("GCG" software, Genetics Computer Group, Inc, Madison Wis.). As examples, SEQ ID NO:2 discloses an ambiguity-maximized p105 coding sequence encompassing all possible nucleic acids encoding the full-length p105 protein. SEQ ID NO:3 discloses a natural *human* cDNA sequence encoding p105, SEQ ID NO:4 is a p105 coding sequence codon-optimized for E. coli, SEQ ID NO:5 is a p105 coding sequence codon optimized for mammalian cell expression. *Telomerase* encoding nucleic acids may be part of *human* *telomerase*-expression vectors and may be incorporated into recombinant host cells, e.g. for expression and screening, transgenic animals, e.g. for functional studies such as the efficacy of candidate drugs for disease associated with *human* *telomerase*-mediated signal transduction, etc. Expression systems are selected and/or tailored to effect *human* *telomerase* protein structural and functional variants through alternative post-translational processing.

BSUM(32) The invention also provides nucleic acid hybridization probes and replication/amplification primers having a *human* *telomerase* cDNA specific sequence contained in SEQ ID NO:3, bases 1-2345, and sufficient to effect specific hybridization thereto (i.e. specifically hybridize with SEQ ID NO:3, bases 1-2345 in the presence of natural ciliate *telomerase* cDNA, preferably in the presence of non-mammalian *telomerase* cDNA and more preferably, in the presence of *murine* *telomerase* cDNA). Demonstrating specific hybridization generally requires stringent conditions, for example, hybridizing in a buffer comprising 30% formamide in 5.times.SSPE (0.18M. . . temperature of 42° C. and remaining bound when subject to washing at 42° C. with 0.2.times.SSPE buffer at 42° C. *Human* *telomerase* cDNA homologs can also be distinguished from other protein using alignment algorithms, such as BLASTX (Altschul et al. (1990) Basic. . .

BSUM(33) The invention also provides non-natural sequence, recombinant and isolated natural sequence *human* *telomerase* RNA. Natural *human* *telomerase* RNA sequences include the nucleic acid disclosed as SEQ ID NO:6, or a fragment thereof sufficient to specifically hybridize with. . . et al. 1995, Science 269, 1236-1241. Such fragments necessarily distinguish the previously described (Feng et al. 1995, Science 269, 1236-1241) *human* RNA species. Preferred such fragments comprise SEQ ID NO:6, bases 191-210, bases 245-259, bases 341-369 or bases 381-399. Non-natural sequences. . . mutations of SEQ ID NO:6, where such derivatives/mutations provide alteration in template, protein binding, or other regions to effect altered *telomerase* substrate specificity or altered reaction product (e.g. any predetermined sequence), etc.; see, e.g. Autexier et al., 1994, Genes & Develop. . . DNA Replication, DePamphilis, Ed., Cold Spring Harbor Laboratory Press. Additional

derivatives function as dominant negative fragments which effectively compete for "telomerase" assembly. For examples, SEQ ID NO:7, 8 and 9 are derivatives which provide for modified substrate specificity and polymerase reaction. . .

BSUM(34)The . . . applications including use as translatable transcripts, hybridization probes, PCR primers, diagnostic nucleic acids, etc.; use in detecting the presence of "human" "telomerase" genes and gene transcripts and in detecting or amplifying nucleic acids encoding additional "human" "telomerase" homologs and structural analogs.

BSUM(35) In diagnosis, "human" "telomerase" hybridization probes find use in identifying wild-type and mutant "human" "telomerase" alleles in clinical and laboratory samples. Mutant alleles are used to generate allele-specific oligonucleotide (ASO) probes for high-throughput clinical diagnoses. In therapy, therapeutic "human" "telomerase" nucleic acids are used to modulate cellular expression or intracellular concentration or availability of active "telomerase". A wide variety of indications may be treated, either prophylactically or therapeutically with the subject compositions. For example, where limitation of cell growth is desired, e.g. neoproliferative disease, a reduction in "telomerase" [redacted]ession is effected by introducing into the targeted cell type [redacted]an" "telomerase" nucleic acids which reduce the functional expression of "human" "telomerase" gene products (e.g. nucleic acids capable of inhibiting translation of a functional "telomerase" transcript). Conditions for treatment include various cancers, where any of a wide variety of cell types may be involved, restenosis,. . .

BSUM(36) "Telomerase" inhibitory nucleic acids are typically antisense: single-stranded sequences comprising complements of the disclosed natural "telomerase" coding sequences. Antisense modulation of the expression of a given "telomerase" protein may employ "telomerase" antisense nucleic acids operably linked to gene regulatory sequences. Cell are transfected with a vector comprising a "human" "telomerase" sequence with a promoter sequence oriented such that transcription of the gene yields an antisense transcript capable of binding to endogenous "human" "telomerase" protein encoding mRNA. Transcription of the antisense nucleic acid may be constitutive or inducible and the vector may provide for. . . stable extrachromosomal maintenance or integration. Alternatively, single-stranded antisense nucleic acids that bind to genomic DNA or mRNA encoding a given "human" "telomerase" protein may be administered to the target cell, in or temporarily isolated from a host, at a concentration that results. . .

BSUM(37) In . . . certain hypersensitivities, atrophic diseases, etc., an increase in cell growth or proliferation is desired. In these [redacted]ations, an enhancement in "human" "telomerase" expression is [redacted]ted by introducing into the targeted cell type "human" "telomerase" nucleic acids which increase the functional expression of "human" "telomerase" gene products. Conditions for treatment include multiple sclerosis, where certain neuronal cells are involved, inflammatory disease states such as rheumatoid. . . diseases such as HIV infection where certain uninfected host cells are involved, or the like. Such nucleic acids may be "human" "telomerase" expression vectors, vectors which upregulate the functional expression of an endogenous "human" "telomerase" allele, or replacement vectors for targeted correction of "human" "telomerase" mutant alleles.

BSUM(39) The . . . of host production cells in culture. Specifically, cultured cells are transfected with nucleic acids which effect the up-regulation of endogenous "telomerase" or the expression of an exogenous "telomerase". For example, nucleic acids encoding functional "human" "telomerase" operably linked to a transcriptional promoter are used to over-express the exogenous "telomerase" in the host cell. "Telomerase"-expressing cells demonstrate enhanced survival ability at elevated cell densities and over extended culture periods.

BSUM(40) The invention provides efficient methods of identifying agents, compounds or lead compounds for agents active at the level of a "human" "telomerase" modulatable cellular function. Generally, these screening methods involve assaying for compounds which modulate "human" "telomerase" interaction with a natural "human" "telomerase" binding target. A wide variety of assays for binding agents are provided including labeled in vitro telomere polymerase assays, protein-protein. . . high throughput screening of chemical libraries for lead compounds. Identified reagents find use in the pharmaceutical industries for animal and "human" trials; for example, the reagents may be derivatized and rescreened in in vitro and in vivo assays to optimize activity. . .

BSUM(41) In vitro binding assays employ a mixture of components including a "human" "telomerase" protein, which may be part of multi-subunit "telomerase", a fusion product with another peptide or polypeptide, e.g. a tag for detection or anchoring, etc. The assay mixtures comprise a natural intracellular "human" "telomerase" binding target, e.g. a substrate. While native binding targets may be used, it is frequently preferred to use portions (e.g. peptides, nucleic acid fragments) thereof so long as the portion provides binding affinity and avidity to the subject "human" "telomerase" conveniently measurable in the assay. The assay mixture also comprises a candidate pharmacological agent. Candidate agents encompass numerous chemical classes,. . .

BSUM(42) The resultant mixture is incubated under conditions whereby, but for the presence of the candidate pharmacological agent, the "human" "telomerase" specifically binds the cellular binding target, portion or analog with a reference binding affinity. The mixture components can be added. . .

BSUM(43) After incubation, the agent-biased binding between the "human" "telomerase" and one or more binding targets is detected by any convenient way. For cell-free binding type assays, a separation step. . . (e.g. gel filtration, affinity, etc.). For telomere polymerase assays, binding is detected by a change in the polymerization by the "telomerase" of a nucleic acid or nucleic acid analog on the substrate.

BSUM(45) A difference in the binding affinity of the "human" "telomerase" protein to the target in the absence of the agent as compared with the binding affinity in the presence of the agent indicates that the agent modulates the binding of the "human" "telomerase" protein to the "human" "telomerase" binding target. Analogously, in the cell-based transcription assay also described below, a difference in the "human" "telomerase" transcriptional induction in the presence and absence of an agent indicates the agent modulates "human" "telomerase"-induced transcription. A difference, as used herein, is statistically significant and preferably represents at least a 50%, more preferably at least. . .

DETD(5) "human" "telomerase": 10^{-8} -10^{-5} M "human" "telomerase" in PBS.

DETD(18) Add 40 μl "human" "telomerase" (1-1000 fmoles/40 ul in assay buffer)

DETD(30)2. Protocol for High Throughput "Human" "Telomerase" Subunit-RNA Complex Formation Assay

DETD(35) ^{32} P "human" "telomerase" protein 10.times.stock: 10^{-9} -10^{-6} M "cold" "human" "telomerase" subunit (p105) supplemented with 200,000-250,000 cpm of labeled "human" "telomerase" (Beckman counter). Place in the 4° C. microfridge during screening.

DETD(46) Add 10 μl ^{33} P-"human" "telomerase" protein (20,000-25,000 cpm/0.1-10 pmoles/well=10^{-9} -10^{-7} M final concentration).

US PAT NO: 5,583,016 [IMAGE AVAILABLE] L6: 31 of 33
BSUM(3) The present invention relates to "human" "telomerase", a ribonucleoprotein "enzyme" involved in "human" telomere DNA synthesis. The invention provides methods and compositions relating to the fields of molecular biology, chemistry, pharmacology, and medical. ..

BSUM(5) The DNA at the ends or telomeres of the chromosomes of eukaryotes usually consists of tandemly repeated simple sequences. "Telomerase" is a ribonucleoprotein "enzyme" that synthesizes one strand of the telomeric DNA using as a template a sequence contained within the RNA component of the "enzyme". See Blackburn, 1992, Annu. Rev. Biochem. 61:113-129, incorporated herein by reference.

BSUM(6) The RNA component of "human" "telomerase" has not been reported in the scientific literature to date, although "human" "telomerase" is known to synthesize telomeric repeat units with the sequence 5'-TTAGGG-3'. See Morin, 1989, Cell. 59:521-529, and Morin, 1991, Nature. . . been sufficient to enable the isolation and identification of the remainder of the nucleotide sequence of the RNA component of "human" "telomerase". The RNA component of the "telomerase" "enzymes" of Saccharomyces cerevisiae, certain species of Tetrahymena, as well as that of other ciliates, such as Euplotes and Glaucoma, has. . . Blackburn, 1991, Cell 67:343-353; and Shippen-Lentz and Blackburn, 1990, Science 247:546-552, each of which is incorporated herein by reference. The "telomerase" "enzymes" of these ciliates synthesize telomeric repeat units distinct from that in humans.

BSUM(7) There is a great need for more information about "human" "telomerase". Despite the seemingly simple nature of the repeat units of telomeric DNA, scientists have long known that telomeres have an. . . speculated that loss of telomeric DNA may act as a trigger of cellular senescence and aging and that regulation of "telomerase" may have important biological implications. See Harley, 1991, Mutation Research 256:271-282, incorporated herein by reference.

BSUM(11) In . . . first aspect, the present invention provides the RNA component of, as well as the gene for the RNA component of, "human" "telomerase" in substantially pure form, as well as nucleic acids comprising all or at least a useful portion of the nucleotide sequence of the RNA component of "human" "telomerase". The present invention also provides RNA component nucleic acids from other species, which nucleic acids share substantial homology with the RNA component of "human" "telomerase", including but not limited to, the RNA components of mammals, such as primates. Other useful nucleic acids of the invention. . . component and which interact with the RNA component or the gene for the RNA component or the protein components of "human" "telomerase" in a useful way; and nucleic acids that do not share significant sequence homology or complementarity to the RNA component. . .

BSUM(12) Thus, . . . triple helix-forming oligonucleotide, or other oligonucleotide that can be used in vivo or in vitro to inhibit the activity of "human" "telomerase". Such oligonucleotides can block "telomerase" activity in a number of ways, including by preventing transcription of the "telomerase" gene (for instance, by triple helix formation) or by binding to the RNA component of "telomerase" in a manner that prevents a functional ribonucleoprotein "telomerase" from assembling or prevents the RNA component, once assembled into the "telomerase" "enzyme" complex, from serving as a template for telomeric DNA synthesis. Typically, and depending on mode of action, these oligonucleotides of. . . or more nucleotides that is either identical or complementary to a specific sequence of nucleotides in the RNA component of "telomerase" or the gene for the RNA component of "telomerase".

BSUM(13) Another type of useful nucleic acid of the invention is a ribozyme able to cleave specifically the RNA component of "human" "telomerase", rendering the "enzyme" inactive. Yet another type of useful nucleic acid of the invention is a probe or primer that binds specifically to the RNA component of "human" "telomerase" and so can be used, e.g., to detect the presence of "telomerase" in a sample. Finally, useful nucleic acids of the invention include recombinant expression plasmids for producing the nucleic acids of the invention. One especially useful type of such a plasmid is a plasmid used for "human" gene therapy. Useful plasmids of the invention for "human" gene therapy come in a variety of types, including not only those that encode antisense oligonucleotides or ribozymes but also those that drive expression of the RNA component of "human" "telomerase" or a deleted or otherwise altered (mutated) version of the RNA component of "human" (or other species with RNA component sequences substantially homologous to the "human" RNA component) "telomerase" or the gene for the same.

BSUM(14) In a second aspect, the invention provides methods for treating a condition associated with the "telomerase" activity within a cell or group of cells by contacting the cell(s) with a therapeutically effective

amount of an agent that alters "telomerase" activity in that cell. Such agents include the "telomerase" RNA component-encoding nucleic acids, triple helix-forming oligonucleotides, antisense oligonucleotides, ribozymes, and plasmids for "human" gene therapy described above. In a related aspect, the invention provides pharmaceutical compositions comprising these therapeutic agents together with a. . .

BSUM(15) In a third aspect, the invention provides diagnostic methods for determining the level, amount, or presence of the RNA component of "human" "telomerase", "telomerase", or "telomerase" activity in a cell, cell population, or tissue sample, or an extract of any of the foregoing. In a related. . .

BSUM(16) In a fourth aspect, the present invention provides recombinant "telomerase" preparations and methods for producing such preparations. Thus, the present invention provides a recombinant "human" "telomerase" that comprises the protein components of "human" "telomerase" as well as the protein components of "telomerase" from a mammalian species with an RNA component substantially homologous to the RNA component of "human" "telomerase" in association with a recombinant RNA component of the invention. Such recombinant RNA co[redacted]nt molecules of the invention include those. . . to a naturally o[redacted] RNA component molecule that are produced in recombinant host cells. The method for producing such recombinant "telomerase" molecules comprises transforming a eukaryotic host cell that expresses the protein components of "telomerase" with a recombinant expression vector that encodes an RNA component molecule of the invention, and culturing said host cells transformed. . . said vector under conditions such that the protein components and RNA component are expressed and assemble to form an active "telomerase" molecule capable of adding sequences (not necessarily the same sequence added by native "telomerase") to telomeres of chromosomal DNA.

BSUM(17) In a fifth aspect, the invention provides methods for purifying the protein components of "human" "telomerase" as well as the protein components of "telomerase" from a mammalian species with an RNA component substantially homologous to the RNA component of "human" "telomerase". The present invention also provides methods for isolating and identifying nucleic acids encoding such protein components. In related aspects, the present invention provides purified "human" "telomerase" and purified "telomerase" of mammalian species with an RNA component substantially homologous to the RNA component of "human" "telomerase", as well as purified nucleic acids that encode one or more components of such "telomerase" preparations. The present invention also provides pharmaceutical compositions comprising as an active ingredient the protein components of "telomerase" or a nucleic acid th[redacted]des or interacts with a nucleic acid that encodes a protein co[redacted]nt of "telomerase".

BSUM(20) The present invention provides methods, reagents, and pharmaceutical compositions relating to the ribonucleoprotein "human" "telomerase". The invention in part arises out of the cloning and isolation of the RNA component of "human" "telomerase" and the gene for that RNA component. The nucleotide sequence of the RNA component of "human" "telomerase" is shown below. For convenience, the sequence is shown using the standard abbreviations for ribonucleotides (A is riboadenine, G is. . .

BSUM(22) The cloning of the RNA component of "human" "telomerase" required a novel method involving negative selection and cycles of positive selection, described below. Initially, however, an attempt was made. . . amplification. The reverse transcription reaction was initiated with a primer identical to the repeat unit in the single-strand portion of "human" telomeric DNA and thus complementary to a sequence believed to be present in the RNA component of "human" "telomerase". The primer also comprised, at its 5'-end, a sequence corresponding to a restriction "enzyme" recognition site. However, when the cDNA produced by the reverse transcription reaction and PCR amplification was examined by gel electrophoresis. . .

BSUM(23)

The successful cloning effort began with the preparation of cDNA from purified preparations of "human" "telomerase" as well as from cell lines that have "human" "telomerase" activity and from cell lines that do not have detectable "human" "telomerase" activity. The method used to prepare the cDNA is described in detail in Example 1, below. Two negative selection steps and successive cycles of positive selection were used in conjunction with the cDNA preparations from the two "human" cell lines to lower the concentration of unwanted sequences and to raise the concentration of the desired RNA component sequences.

BSUM(24)

The negative selection steps involved the preparation of biotinylated PCR product from cDNA prepared from a "human" cell line that does not have detectable "telomerase" activity. The biotinylated PCR product was denatured and then rehybridized in a solution comprising a much lower concentration of non-biotinylated PCR product (100 biotinylated product: 1 non-biotinylated product) from cDNA prepared from a "human" cell line that does have "telomerase" activity. Given the possibility that the "telomerase" negative cell line might contain some low amount of the RNA component, the hybridization step was conducted to discriminate or. . . binding to streptavidinylated magnetic particles; the supernatant remaining after particle collection contained the desired cDNA for the RNA component of "human" "telomerase". The process for PCR amplification of cDNA is described in Example 2, below.

BSUM(25)

This . . . selection. In the positive selection step, a biotinylated probe complementary to the predicted template sequence in the RNA component of "human" "telomerase" was hybridized to PCR product from an enriched (by negative selection) sample of the PCR-amplified cDNA from a "human" cell line that has "telomerase" activity. After hybridization, the probe/target complexes were bound to avidinylated magnetic beads, which were then collected and used as a. . .

BSUM(26)

After . . . acids were then eluted from the gel sections and amplified by PCR. The PCR amplification products were digested with restriction "enzyme" NotI and then inserted by ligation into the NotI site of plasmid pBluescriptIISK+, commercially available from Stratagene. The resulting plasmids. . . were prepared and hybridized to a probe comprising a telomeric repeat sequence and therefore complementary to the RNA component of "human" "telomerase". A number of clones positive by this test were then analyzed by DNA sequencing and a variety of other tests.

BSUM(27)

These other tests included the following: (1) determination whether antisense oligonucleotides complementary to the putative RNA component would inhibit "telomerase" activity in "human" cell extracts known to contain "telomerase"; (2) determination whether PCR primers specific for a putative RNA component clone sequence could be used to amplify a nucleic acid present in a "telomerase" sample and whether the product observed, if any, would track "telomerase" activity during purification of "telomerase"; and (3) determination whether PCR primers specific for a putative RNA component clone sequence could be used to amplify a nucleic acid present in greater abundance in cell extracts from cells in which "telomerase" activity is known to be high (i.e., tumor cells) than in cell extracts from cells known to produce no or only low amounts of "telomerase" activity. One clone, designated plasmid pGRN7, produced results in these tests consistent with the determination that the plasmid comprised cDNA corresponding to the RNA component of "human" "telomerase".

BSUM(29)

The above results provided convincing evidence that the RNA component of "human" "telomerase" had been cloned, so plasmid pGRN7 was then used to isolate a genomic clone for the RNA component from a "human" cell line, as described in Example 7, below. The genomic clone was identified in and isolated from a genomic library of "human" DNA inserted into a lambda vector FIXII purchased from Stratagene. The desired clone comprising the RNA component gene sequences contained. . . localized to the distal end of the q arm of chromosome 3. The sequence information obtained from a SauIIIA1 restriction "enzyme" recognition site at one end of the .about.15 kb insert to an internal HindIII restriction "enzyme" recognition site, which comprises all of the mature RNA component sequence as well as transcription control elements of the RNA. . .

BSUM(30)

The plasmids described above that were constructed during the cloning of the RNA component of "human" "telomerase" and the gene for the RNA component are important aspects of the present invention. These plasmids can be used to produce the RNA component of, as well as the gene for, "human" "telomerase" in substantially pure form, yet another important aspect of the present invention. In addition, those of skill in the art. . . pure form, that comprise all or at least a useful portion of the nucleotide sequence of the RNA component of "human" "telomerase" are useful materials provided by the present invention.

BSUM(32)

Just . . . the invention is an antisense oligonucleotide that can be used in vivo or in vitro to inhibit the activity of "human" "telomerase". Antisense oligonucleotides comprise a specific sequence of from about 10 to about 25 to 200 or more (i.e., large enough. . . delivery, to administer in vivo, if desired) nucleotides complementary to a specific sequence of nucleotides in the RNA component of "human" "telomerase". The mechanism of action of such oligonucleotides can involve binding of the RNA component either to prevent assembly of the functional ribonucleoprotein "telomerase" or to prevent the RNA component from serving as a template for telomeric DNA synthesis.

BSUM(33)

Illustrative antisense oligonucleotides of the invention that serve to inhibit "telomerase" activity in vivo and/or in vitro include the oligonucleotides mentioned above in connection with the tests to determine whether clone pGRN7 comprised the cDNA for the RNA component of "human" "telomerase". Three such oligonucleotides were synthesized as 2'-O-methyl RNA oligonucleotides, which bind more tightly to RNA than DNA oligonucleotides and are more resistant to hydrolysis than unmodified RNA oligonucleotides, and, as noted above, were used to demonstrate inhibition of "telomerase" activity in vitro. The sequence of each of these O-methyl RNA oligonucleotides is shown below. ##STR3## These oligonucleotides can also be used to inhibit "telomerase" activity in "human" cells.

BSUM(36)

In . . . helix-forming oligonucleotides of the invention, "sense" oligonucleotides identical in sequence to at least a portion of the RNA component of "human" "telomerase" can also be used to inhibit "telomerase" activity. Oligonucleotides of the invention of this type are characterized in comprising either (1) less than the complete sequence of the RNA component needed to form a functional "telomerase" "enzyme" or (2) the complete sequence of the RNA component needed to form a functional "telomerase" "enzyme" as well as a substitution or insertion of one or more nucleotides that render the resulting RNA non-functional. In both cases, inhibition of "telomerase" activity is observed due to the "mutant" RNA component binding the protein components of "human" "telomerase" to form an inactive "telomerase" molecule. The mechanism of action of such oligonucleotides thus involves the assembly of a non-functional ribonucleoprotein "telomerase" or the prevention of assembly of a functional ribonucleoprotein "telomerase". Sense oligonucleotides of the invention of this type typically comprise a

specific sequence of from about 20, 50 200, 400, 500, or more nucleotides identical to a specific sequence of nucleotides in the RNA component of "human" "telomerase".

BSUM(37)

Thus, another useful oligonucleotide of the invention comprises an altered or mutated sequence of the RNA component of "human" "telomerase". Yu et al., 1990, Nature 344: 126, shows that a mutated form of the RNA component of Tetrahymena "telomerase" can be incorporated into the "telomerase" of Tetrahymena cells and that the incorporation has deleterious effects on those cells. Incorporation of mutated forms of the RNA component of "human" "telomerase" may have similar effects on "human" cells that otherwise have "telomerase" activity without affecting normal "human" cells that do not have "telomerase" activity. Such mutated forms include those in which the sequence 5'-CTAACCCTA-3' [SEQ ID NO: 8] is mutated to 5'-CAAACCCAA-3' [SEQ. . . units incorporated into the chromosomal DNA, thus affecting chromosome structure and function. Such oligonucleotides can be designed to contain restriction "enzyme" recognition sites useful in diagnostic methods for the presence of the altered RNA component via restriction "[redacted] digestion of telomeric DNA or an extended "telomerase" substrate.

BSUM(39)

The assays showed that the "telomerase" activity in the cells resulted in the formation of nucleic acids comprising the altered sequences, indicating that the genomic clone. . . that the plasmids comprised an altered but functional RNA component gene. These results illustrate how the present invention provides recombinant "telomerase" preparations and methods for producing such preparations. The present invention provides a recombinant "human" "telomerase" that comprises the protein components of "human" "telomerase" in functional association with a recombinant RNA component of the invention. Such recombinant RNA component molecules of the invention include. . . to a naturally occurring RNA component molecule that are produced in recombinant host cells. The method for producing such recombinant "telomerase" molecules comprises transforming a eukaryotic host cell that expresses the protein components of "telomerase" with a recombinant expression vector that encodes an RNA component molecule of the invention, and culturing said host cells transformed. . . said vector under conditions such that the protein components and RNA component are expressed and assemble to form an active "telomerase" molecule capable of adding sequences (not necessarily the same sequence added by native "telomerase") to telomeres of chromosomal DNA. Other useful embodiments of such recombinant DNA expression vectors (or plasmids) include plasmids that comprise the gene for the RNA component of "human" "telomerase" with a deletion, insertion, or other modification that renders the gene non-functional. Such plasmids are especially useful for "human" gene therapy to "knock-out" the endogenous RNA component gene, although a highly efficient transformation and recombination system is required, to. . .

BSUM(40)

Other oligonucleotides of the invention called "ribozymes" can also be used to inhibit "telomerase" activity. Unlike the antisense and other oligonucleotides described above, which bind to an RNA, a DNA, or a "telomerase" protein component, a ribozyme not only binds but also specifically cleaves and thereby potentially inactivates a target RNA, such as the RNA component of "human" "telomerase". Such a ribozyme can comprise 5'- and 3'-terminal sequences complementary to the "telomerase" RNA. Depending on the site of cleavage, a ribozyme can render the "telomerase" "enzyme" inactive. See PCT patent publication No. 93/23572, supra. Those in the art upon review of the RNA sequence of the "human" "telomerase" RNA component will note that several useful ribozyme target sites are present and susceptible to cleavage by, for example, a. . . the ribozymes below, which are RNA molecules having the sequences indicated: ##STR4## Other optimum target sites for ribozyme-mediated inhibition of "telomerase" activity can be determined as described by Sullivan et al., PCT patent publication No. 94/02595 and Draper et al., PCT. . .

BSUM(44)

Other therapeutic methods of the invention employ the "telomerase" RNA nucleic acid of the invention to stimulate "telomerase" activity and to extend replicative cell life span. These methods can be carried out by delivering to a cell a functional recombinant "telomerase" ribonucleoprotein of the invention to the cell. For instance, the ribonucleoprotein can be delivered to a cell in a liposome, or the gene for the RNA component of "human" "telomerase" (or a recombinant gene with different regulatory elements) can be used in a eukaryotic expression plasmid (with or without sequences coding for the expression of the protein components of "telomerase") to activate "telomerase" activity in various normal "human" cells that otherwise lack detectable "telomerase" activity due to low levels of expression of the RNA component or a protein component of "telomerase". If the "telomerase" RNA component is not sufficient to stimulate "telomerase" activity, then the RNA component can be transfected along with genes expressing the protein components of "telomerase" to stimulate "telomerase" activity. Thus, the invention provides methods for treating a condition associated with the "telomerase" activity within a cell or group of cells by contacting the cell(s) with a therapeutically effective amount of an agent that alters "telomerase" activity in that cell.

BSUM(50)

In related aspects, the invention features pharmaceutical compositions including a therapeutically effective amount of a "telomerase" inhibitor or "telomerase" activator of the invention. Pharmaceutical compositions of "telomerase" inhibitors of the invention include a mutant RNA component of "human" "telomerase", an antisense oligonucleotide or triple helix-forming oligonucleotide that binds the RNA component or the gene for the same of "human" "telomerase", or a ribozyme able to cleave the RNA component of "human" "telomerase", or combinations of the same or other pharmaceuticals in a pharmaceutically acceptable carrier or salt. Other pharmaceutical compositions of the invention comprise a "telomerase" activator preparation, such as purified "human" "telomerase" or mRNA for the protein components of "telomerase" and the RNA component of "telomerase", and are used to treat senescence-related disease. The therapeutic agent can be provided in a formulation suitable for parenteral, nasal,. . .

BSUM(51)

The . . . methods described above. The invention provides diagnostic methods for determining the level, amount, or presence of the RNA component of "human" "telomerase", "telomerase", or "telomerase" activity in a cell, cell population, or tissue sample. In a related aspect, the present invention provides useful reagents for. . . above in connection with the tests conducted to determine that clone pGRN7 contained the cDNA for the RNA component of "human" "telomerase", the levels of the RNA component are elevated in tumor cells. Thus, detection of the RNA component is a useful. . .

BSUM(52)

In addition, probes or primers that bind specifically to the RNA component of "human" "telomerase" (or either strand of the gene for the same) can be used in diagnostic methods to detect the presence of "telomerase" nucleic acid in a sample. Primers and probes are oligonucleotides that are complementary, and so will bind, to a target. . .

BSUM(54)

Depending . . . the length and intended function of the primer, probe, or other nucleic acid comprising sequences from the RNA component of "human" "telomerase", expression plasmids of the invention may be useful. For instance, recombinant production of the full-length RNA component of the invention. . .

BSUM(55)

The . . . in the 5'-region of the gene, and RNA component coding region, can be used to express the RNA component in "human" cells, including "human" cells that have been immortalized by viral transformation or cancer. The promoter of the RNA component gene may be regulated,. . . component gene to a coding sequence for a "reporter" coding sequence, such as the coding sequence for beta-galactosidase or another "enzyme" or protein the expression of which can be readily monitored. Thus, the promoter and other regulatory elements of the gene for the RNA component of "human" "telomerase" can be used not only to express the RNA component but also protein components of "human" "telomerase", antisense or other oligonucleotides, as well as other gene products of interest in "human" cells. Expression plasmids comprising the intact gene for the RNA component of "human" "telomerase" can be especially useful for a variety of purposes, including gene therapy. Those of skill in the art recognize that. . .

BSUM(56)

As indicated by the foregoing description, access to purified nucleic acids comprising the sequence of the RNA component of "human" "telomerase" provides valuable diagnostic and therapeutic methods and reagents, as well as other important benefits. One important benefit of the present. . . and reagents of the invention can be used to isolate the RNA component and genes for the RNA component of "telomerase" from any mammalian species that has an RNA component substantially homologous to the "human" RNA component of the present invention. The phrase "substantially homologous" refers to that degree of homology required for specific hybridization of an oligonucleotide or nucleic acid sequence of the "human" RNA component to a nucleic acid sequence of an RNA component sequence of another mammalian species. Given such substantial homology,. . .

BSUM(57)

For . . . these and other similar techniques, those of ordinary skill can readily isolate not only variant RNA component nucleic acids from "human" cells but also homologous RNA component nucleic acids from other mammalian cells, such as cells from primates, from mammals of. . . nucleic acids can be used to prepare transgenic animals of great value for screening and testing of pharmaceuticals that regulate "telomerase" activity. For instance, by using a plasmid of the invention, one can "knock out" the RNA component gene or replace. . . RNA component gene with a recombinant inducible gene in a Mus spretus embryonic stem cell and then generate a transgenic "mouse" that will be useful as a model or test system for the study of age- or senescence-related disease. Example 9,. . .

BSUM(58)

The reagents of the present invention also allow the cloning and isolation of nucleic acids encoding the protein components of "human" as well as other mammalian "telomerase" "enzymes", which have not previously been available. Access to such nucleic acids provide complementary benefits to those provided by the nucleic acids comprising nucleic acid sequences of the RNA component of "human" "telomerase". For instance, and as noted above, the therapeutic benefits of the present invention can be enhanced, in some instances, by use of purified preparations of the protein components of "human" "telomerase" and by access to nucleic acids encoding the same. The nucleic acids of the invention that encode the RNA component of "human" "telomerase" can be used to isolate the nucleic acid encoding the protein components of "human" "telomerase", allowing access to such benefits. Thus, the invention provides methods for isolating and purifying the protein components of "human" "telomerase", as well as for identifying and isolating nucleic acids encoding the protein components of "human" "telomerase". In related aspects, the present invention provides purified "human" "telomerase", purified nucleic acids that encode the

protein components of "human" "telomerase", recombinant expression plasmids for the protein components of "human" "telomerase". The invention also provides pharmaceutical compositions comprising as an active ingredient either the protein components of "human" "telomerase" or a nucleic acid that either encodes those protein components or interacts with nucleic acids that encode those protein components,. . .

BSUM(59)

The cloned RNA component of "human" "telomerase" can be used to identify and clone nucleic acids encoding the protein components of the ribonucleoprotein "telomerase" "enzyme". Several different methods can be employed to achieve identification and cloning of the protein components. For instance, one can use affinity capture of the "enzyme" or partially denatured "enzyme" using as an affinity ligand either (1) nucleotide sequences complementary to the RNA component to bind to the RNA component of the intact "enzyme"; or (2) the RNA component to bind the protein components of a partially or fully denatured "enzyme". The ligand can be affixed to a solid support or chemically modified (e.g., biotinylated) for subsequent immobilization on the support. Exposure of [redacted]xtracts containing "human" "telomerase", followed by washing [redacted]ution of the "telomerase" "enzyme" bound to the support, provides a highly purified preparation of the "telomerase" "enzyme". The protein components can then be optionally purified further or directly analyzed by protein sequencing. The protein sequence determined can. . . cloning the cDNA or identifying a clone in a genomic bank comprising nucleic acids that encode a protein component of "telomerase".

BSUM(60)

Affinity capture of "telomerase" utilizing an engineered RNA component can also be conducted using in vitro transcribed "telomerase" RNA and a system for the reconstitution of "telomerase" "enzyme" activity. See Autexier and Greider, 1994, Genes & Development 8:563-575, incorporated herein by reference. The RNA is engineered to contain. . . sequence-specific nucleic acid binding protein, or an organic dye that binds tightly to a specific RNA sequence. The tolerance of "telomerase" for the tag sequence and position can be tested using standard methods. Synthesis of the altered RNA component and the. . . out in vivo. Affinity capture using the immobilized ligand for the RNA tag can then be used to isolate the "enzyme".

BSUM(61)

[redacted]ssion screening can also be used to isolate the protein components [redacted] telomerase" "enzyme". In this method, cDNA expression libraries can be screened with labeled "telomerase" RNA, and cDNAs encoding proteins that bind specifically to "telomerase" RNA can be identified. A molecular genetic approach using translational inhibition can also be used to isolate nucleic acids encoding the protein components of the "telomerase" "enzyme". In this method, "telomerase" RNA sequences will be fused upstream of a selectable marker. When expressed in a suitable system, the selectable marker will be functional. When cDNA encoding a "telomerase" RNA binding protein is expressed, the protein will bind to its recognition sequence thereby blocking translation of the selectable marker,. . .

BSUM(62)

"Telomerase" RNA binding or "telomerase" activity assays for detection of specific binding proteins and activity can be used to facilitate the purification of the "telomerase" "enzyme" and the identification of nucleic acids that encode the protein components of the "enzyme". For example, nucleic acids comprising RNA component sequences can be used as affinity reagents to isolate, identify, and purify peptides,. . . or other compounds that bind specifically to a sequence contained within the RNA component, such as the protein components of "human" "telomerase". Several different formats are available, including gel shift, filter binding, footprinting, Northwestern (RNA probe of protein blot), and photocrosslinking, to. . .

BSUM(63)

As . . . to those of skill in the art upon reading of this disclosure, the present invention provides valuable reagents relating to "human" "telomerase", as well as a variety of useful therapeutic and diagnostic methods. The above description of necessity provides a limited sample. . .

DETD(1)

The . . . to illustrate the invention and provide a description of the methods used to isolate and identify the RNA component of "human" "telomerase" for those of skill in the art. The examples should not be construed as limiting the invention, as the examples. . .

DETD(21)

For the positive selection step of the cyclic selection process used to clone the RNA component of "human" "telomerase", about 2 μg of the PCR-amplified cDNA were diluted into 25 μl of TE buffer and then mixed with 1.25. . . cyclic selection process is functioning properly with respect to the molecule of interest, in this case the RNA component of "human" "telomerase".

DETD(35)

Cloning the gene for the RNA component of "human" "telomerase"

DETD(36)

The procedures used to clone the gene for the RNA component of "human" "telomerase" were carried out as generally described in Maniatis et. al., Laboratory Molecular Cloning Manual. A genomic DNA library of DNA from the "human" lung fibroblast cell line WI-38 inserted into phage lambda vector FIXII was purchased from Stratagene. The phage were plated at. . .

DETD(38)

One strong signal emanated from the filter containing a phage, later designated 28-1, comprising the gene for the RNA component of "human" "telomerase". The plaque corresponding to the signal observed on the filter was used to make secondary plates, so that an isolated. . . comprises several restriction fragments that contain sequences that hybridize with RNA component sequences on pGRN7: a 4.2 kb EcoRI restriction "enzyme" fragment; a 4.2 kb ClaI restriction "enzyme" fragment, and a 2.5 kb HindIII-SacI restriction "enzyme" fragment. The latter fragment comprises the entire .about.560 nucleotide sequence of the RNA component shown above and is believed to comprise the complete gene for the RNA component. The plasmid comprising the 2.5 kb HindIII-SacI restriction "enzyme" fragment in the pBluescript vector was designated plasmid pGRN33 and is available from the American Type Culture Collection under the accession No. ATCC 75926. To the extent the "human" gene may comprise sequences other than those on the 2.5 kb fragment, those sequences can be isolated from phage 28-1. . .

DETD(42)

Antisense plasmids for the RNA component of "human" "telomerase"

DETD(43)

Antisense . . . plasmid comprises puromycin resistance-conferring, DHFR, and hygromycin B resistance-conferring genes as selectable markers, the SV40 origin of replication; the inducible "human" metallothionein gene promoter positioned for expression of the antisense strand of the gene for the RNA component of "human" "telomerase" (one could also use a stronger promoter to get higher expression levels), and the SV40 late poly A addition site.

DETD(44)

The . . . the fibrosarcoma cell line HT1080. HT1080 cells are normally immortal; expression of the antisense RNA for the RNA component of "human" "telomerase" should prevent the RNA component of "human" "telomerase" from association with the protein components, blocking the formation of active "telomerase" and rendering the cells mortal.

DETD(48)

For . . . from each of these mammalian species can be cloned as described above for the gene for the RNA component of "human" "telomerase".

CLAIMS: We . . . comprising an oligonucleotide having a contiguous sequence of at least 25 nucleotides in a sequence complementary or identical to a "human" genomic DNA sequence encoding the RNA component of "human" "telomerase" located in an .about.2.5 kb HindIII-SacI insert of plasmid pGRN33 (ATCC 75926).

4. . . . isolated and purified recombinant nucleic acid of claim 1 that is complementary to the DNA encoding the RNA component of "human" "telomerase".

17. . . . and purified recombinant nucleic acid of claim 16 wherein said recombinant nucleic acid functions to produce the oligonucleotide in a "human" cell such that the RNA is capable of being assembled by the cell into a functional "telomerase" molecule.

18. The isolated and purified recombinant nucleic acid of claim 10 wherein the oligonucleotide comprises a "human" gene for the RNA component of "human" "telomerase".

22. . . . between 25 and 1000 nucleotides in length in a sequence identical or complementary to a contiguous sequence contained within a "human" genomic DNA sequence "encoding the RNA component of "human" "telomerase"" located in an .about.2.5 kb HindIII-SacI insert of plasmid pGRN33 (ATCC 75926).

31. Isolated RNA component of "human" "telomerase".

33. . . . comprising an oligonucleotide having a contiguous sequence of at least 25 nucleotides in a sequence complementary or identical to a "human" genomic DNA sequence encoding the RNA component of "human" "telomerase" located in an .about.2.5 kb HindIII-Sac I insert of plasmid pGRN33 (ATCC 75926).

38. . . . cell of claim 37 wherein the recombinant nucleic acid encodes an RNA molecule that can associate with protein components of "human" "telomerase" to produce "telomerase" activity capable of adding sequences of repeating units of nucleotides to telomeres.

44. A method for producing the RNA component of "human" "telomerase" comprising the step of culturing a eukaryotic host cell transformed with a recombinant nucleic acid comprising a promoter positioned to drive the transcription of an oligonucleotide encoding an RNA component of "human" "telomerase".

45. The method of claim 44 wherein the oligonucleotide encoding the RNA component of "human" "telomerase" includes a sequence from a "human" genomic DNA sequence located in an .about.2.5 kb HindIII-SacI insert of plasmid pGRN33 (ATCC 75926).

47. A method for producing a recombinant "telomerase" "enzyme", said method comprising transforming a eukaryotic host cell capable of expressing protein components of "telomerase", with a recombinant nucleic acid comprising a promoter positioned to drive the transcription of an oligonucleotide encoding the RNA component of "human" "telomerase", said recombinant nucleic acid functioning to produce the

oligonucleotide in a eukaryotic cell, and culturing said host cells transformed with. . . said vector under conditions such that the protein components and RNA component are expressed and assemble to form an active "telomerase" molecule capable of adding sequences to telomeres of chromosomal DNA.

48. . . . method of claim 47 wherein the RNA has a sequence identical to a contiguous sequence encoding the RNA component of "human" "telomerase" from a "human" genomic DNA sequence located in an .about.2.5 kb HindIII-SacI insert of plasmid pGRN33 (ATCC 75926).

L8
1. 5,721,118, Feb. 24, 1998, Mammalian artificial chromosomes and methods of using same; Immo E. Scheffler, 435/69.1, 320.1, 325, 449; 514/44; 536/23.1, "23.5" [IMAGE AVAILABLE]

2. 5,674,996, Oct. 7, 1997, Cell cycle checkpoint genes; Leland H. Hartwell, et al., 536/24.31, "23.5" [IMAGE AVAILABLE]

US PAT NO: 5,721,118 [IMAGE AVAILABLE] L8: 1 of 2
US-CL-CURRENT: 435/69.1, 320.1, 325, 449; 514/44; 536/23.1, "23.5"
D[redacted]8)
F[redacted]itation of chromosomes, minichromosomes or a MAC using "telomerase" associated truncation also can be used to produce a MAC or reduce the size of a MAC. For example, a. . .

US PAT NO: 5,674,996 [IMAGE AVAILABLE] L8: 2 of 2
US-CL-CURRENT: 536/24.31, "23.5"
BSUM(42) The . . . of human chromosome 19p13.3. Since telomeric regions in chromosomes (teleosomes) are subject to frequent rearrangement from incomplete DNA replication and "telomerase" terminal extension, it is thought highly likely that mapping rearrangements of human checkpoint genes may be useful diagnostically for identifying. . .